University of Leeds Ecology and Global Change Standard Soil Measurements and Laboratory Soil Analysis Methods for Rainfor, AfriTRON and ForestPlots.net

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Contents

1 Introduction

Soil samples collected from plots on behalf of ForestPlots.net are analysed at the School of Geography Laboratories, University of Leeds, UK for eight core variables following standard soil laboratory analysis methods. Other soil analysis may also have been carried out for certain plots and are considered 'extra' soil measurements by ForestPlots.net. The soil analysis protocols are describe in this document.

2 Core Variables

2.1 Particle Size (Soil Texture)
2.2 pH
2.3 Carbon/Nitrogen
2.4 Total Phosphorous
2.5 Macronutrients for Cation, CEC and Metals: Aluminium (Al)
Calcium (Ca)
Potassium (P)
Magnesium (Mg)
Sodium (Na)
2.6 Cations and Cation Exchange Capacity
2.7 Bulk Density
2.8 Loss on Ignition

2.1 Particle Size (Soil Texture)

The mineral fraction of soil is made up of sand, silt and clay. There are several systems of classification for particle sizes used worldwide, below shows the system used by ForestPlots.net:

Soil particle class size

Sand 0.06 – 2 mm
Silt 0.002 – 0.06 mm
Clay <0.002 mm

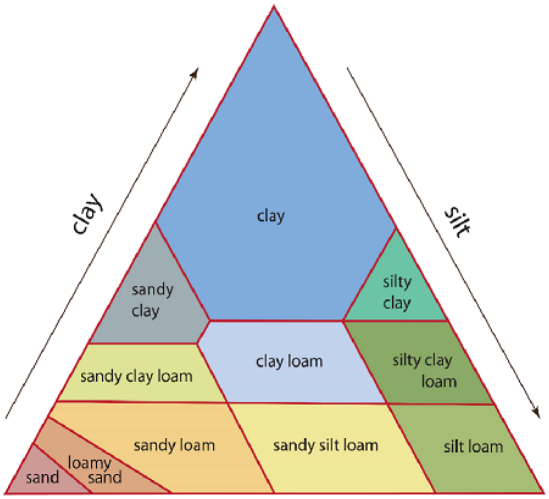
Sand fraction: composed mainly of quartz particles and plays a more passive role in soil function.

Silt fraction: much smaller in size and originate from a mixture of different minerals, such as quartz or micas.

Clay fraction: The smallest soil particles formed from a mixture of minerals. Clay minerals are particularly important because they carry a negative charge, which attracts positively charged nutrient ions, such as calcium, potassium, ammonium and magnesium. Clay minerals therefore provide sites for minerals and nutrients derived from microbial break down of organic matter to stick to. This prevents these nutrients from being washed out of soil in drainage water (leached).

Weathering of clay minerals and the action of microorganisms releases nutrients for plant uptake.

The relative proportion of sand, silt and clay results in texture classes.



Soil pH is a measure of hydrogen ion activity which influences root growth, biological activity and the availability of nutrients to plants. The extent and speed of many chemical reactions and the availability of nutrients are dependent on the pH of soil. Soils with a pH of around 7 have a higher availability of Mg, Ca, K, and N, while Fe, Zn and Cu are less available at high pH.

Cation exchange capacity (CEC) is the total capacity of a soil to hold exchangeable cations. The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic force. This electrical charge is critical to the supply of nutrients to plants because many nutrients exist as cations (e.g. magnesium, potassium and calcium). In general terms, soils with large quantities of negative charge are more fertile because they retain more cations.

2.2 pH

Soil pH is a measure of hydrogen ion activity which influences root growth, biological activity and the availability of nutrients to plants. The extent and speed of many chemical reactions and the availability of nutrients are dependent on the pH of soil. Soils with a pH of around 7 have a higher availability of Mg, Ca, K, and N, while Fe, Zn and Cu are less available at high pH.

2.3 Carbon (C) / Nitrogen (N)

Carbon (C): Soil carbon helps to maintain soil structure by forming stable, larger aggregates that hold plant-available water. Carbon provides substrate and energy to support microbial activity, provides a reservoir of organic N, Phosphorous and other nutrients for plant productivity, and creates physically cohesive soil.

Nitrogen (N): Role: Vital part of proteins and chlorophyll

Primary source: None in soil; fixed from atmosphere by microorganisms.

Availability: Only soluble forms directly available; these are released from organic matter by microorganisms.

2.4 Total Phosphorous

Phosphorus (P)

Role: Key role in energy transfer in cells, DNA, cell membranes.

Primary source: Soil minerals; amount in soil determined by soil parent material.

Availability: Soil minerals rapidly absorb phosphate ions, where they become fixed and unavailable; soil solution concentration very low.

2.5 Macronutrients for Cation, CEC and Metals

Aluminium (Al)

Bonds with phosphorus (P) in a less available and insoluble form in soils and plant roots, thereby creating a P deficiency for plant growth.

Calcium (Ca)

Role: Vital for the growth of new cells especially in roots.

Primary source: Soil minerals; amount in soil determined by soil parent material.

Availability: Rapidly released by available soil minerals and exchange sites; ready available in soils; in most neutral or slightly acid soils calcium dominates soil exchange sites.

Magnesium (Mg)

Role: Constituent of chlorophyll; also important in many enzyme reactions and energy transfer in cells.

Primary source: Soil minerals.

Availability: Rapidly released by available soil minerals and exchange sites; ready available in soils.

Potassium (K)

Role: Main role is in controlling water and ion balance in cells;

Primary source: Soil minerals; amount in soil determined by soil parent material.

Availability: Rapidly released by available soil minerals and exchange sites; ready available in soils.

Sodium (Na)

Role: Sodium is not an essential element for plants but can be used in small quantities, similar to micronutrients, to aid in metabolism and synthesis of chlorophyll.

Primary source: Salts occur naturally within soils and water from natural processes such as mineral weathering

Availability: When salt concentrations in the soil are high, the movement of water from the soil to the root is slowed down. When the salt concentrations in the soil are higher than inside the root cells, the soil will draw water from the root, inhibiting water availability to trees.

2.6 Cation Exchange Capacity (CEC)

Cation exchange capacity (CEC) is the total capacity of a soil to hold exchangeable cations. The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic force.

This electrical charge is critical to the supply of nutrients to plants because many nutrients exist as cations (e.g. magnesium, potassium and calcium). In general terms, soils with large quantities of negative charge are more fertile because they retain more cations.

2.7 Bulk Density

Soil bulk density is defined as the density of the soil as it exists naturally including air spaces and organic matter. Bulk density measurements provide an indication of the ease with which plant roots can penetrate the soil.

2.8 Loss of Ignition

Loss on ignition (LOI) is a widely used methods for measuring organic matter content in soils but does not have a universal standard protocol. A large number of factors may influence its accuracy, such as furnace type, sample mass, duration and temperature of ignition and clay content of samples. LOI can be used to calculate Bulk Density if Bulk Density samples are not taken in the field.

3. Laboratory Methods

3.1 Tropical Soil Sample Preparation

a. Scope

The sample received from the field is oven dried for 48 hours before being prepared for chemical and physical analysis. The sample is firstly ground to <2mm and a sub sample ground further to obtain <500μm for silver-thiourea extraction and ~100μm for elemental analysis.

b. Equipment

Microbiological safety cabinet
Ceramic pestle and mortar
Rubber pestle
Stainless steel sieve 2mm
Stainless steel sieve 500μm
Stainless steel bottom pan
Stainless steel lid
Balance capable of weighing to three decimal places
Containers
Deionised water wash bottle
Oven set to 40°C
Mixer mill with stainless steel grinding jars
Vacuum fitted with a HEPA filter

d. Procedure

Oven drying and preparation of 2mm fraction for pH, particle size and phosphorus analysis

1. Oven dry the samples at 40°C for 48 hours or until you are sure the sample is dry. Periodically mix the soil in the bag to ensure it is completely dry.
2. Transfer the soil to a pre-tared 1 L beaker.
3. Record the weight of the sample to two decimal places.
4. Transfer the samples to a clean tray in order to remove stones/roots prior to chemical/physical analysis. Weigh the stones and roots and record the weight of each to two decimal places.
5. Return roots/stones to the original empty, sample bag.
6. Vacuum the tray in preparation for the next sample.
7. Gently grind the soil to pass through a <2mm stainless steel sieve, cover the sieve with a lid to reduce exposure dust and laboratory contamination. Continue grinding until the entire sample has been processed.
8. Transfer the prepared sample to a pre-labelled self-seal bag. With the sample ID and size classification.

Preparation of 500μm fraction

1. Homogenise (shake the sample bag to mix) the sample.

2. Take a representative sub-sample of approximately ~20 g.
3. Use a rubber pestle to gently aid passing the sample through a 500 μm stainless steel sieve, do not grind the sample.
4. Collect the <500 μm sample that has passed through the sieve and place into a pre-labelled small bag. The bag should be labelled with the sample ID and size classification. The sub-sample is now ready for further preparation. Collect approximately 15 g of sample, sufficient sample for: extraction, duplicate samples and a repeat if necessary.
5. Discard the sample remaining on the sieve that is >500 μm to ensure that the original sample is not biased.
6. Clean the sieves using a sieve brush.
7. Spray the pestle & mortar with Trigene®/Distel® disinfectant, wipe clean with a J-cloth, rinse thoroughly with deionised water, leave to air dry or oven dry. Use a clean pestle & mortar for each sample.
8. Vacuum the work area in between samples to prevent cross contamination.

Preparation of ~100μm fraction for elemental analysis

1. Homogenise (shake the bag to mix) the 2 mm sample fraction sample.
2. Transfer approximately 15 g of sample to a sheet of pre-folded paper.
3. Using the paper as a funnel transfer the sample to a clean, dry grinding jar. The sample jar should be $^{1}/_{3}$ full, adjust the sample amount if necessary.

   NB. Each jar and lid should be a matched pair; check that the number engraved on the lid matches the number on the base.
4. Place the stainless steel ball in the grinding jar.
5. Clamp the jar in the grinder by means of the ratchet fastening. In order to ensure smooth running of the mixer mill, both milling positions must be loaded with approximately the same mass.
6. Start the grinder: 4 minutes at a frequency of 25 Hz.
7. Remove the grinding jar from the grinder and place in the biological safety cabinet, transfer the ground material to a sheet of pre-folded paper.
8. Transfer the ground material to a pre-labelled glassine sample bag. The bag should be labelled with the sample ID and size classification.
9. Soak the grinding jars and balls in a beaker containing Trigene®/Distel® disinfectant, remove as much material as possible using a soft cloth under running water. Do not use abrasives to clean the grinding jar.
10. Sonicate the jars and balls for 1 minute, rinse thoroughly with deionised water.
11. Remove the excess water by shaking the items and transfer to an oven for drying.
12. Vacuum the work area in preparation for the next sample.
13. At the end of the session vacuum and disinfect the working area, surrounding area and equipment, discard any waste material following APHA 006.

3.2 Determination of pH

Introduction

The pH of the soil is potentiometrically measured in the supernatant suspension of a 1:2.5 soil: liquid mixture. The liquid is deionised water (pH-H_2O) for all samples (inc Pit samples) and 1 M KCl solution (pH-KCl) for the Pit samples only.

Equipment

- Top pan balance
- Spatula
- 40 mL containers
- 25 mL measuring cylinder
- Reciprocating shaker
- pH meter
- Deionised water wash bottle
- Magnetic stirrer and beads
- Beaker for waste

Use of buffers

The effect of opening the bottle

Change of the buffer pH is caused by opening the bottle and allowing CO_2 from ambient air to dissolve in buffer solution. Every opening adds CO_2. Tip: Opening the bottle with buffer solution should be as short as possible. The bottle should never be open for a longer time. pH buffer quality directly influences the accuracy of the pH probe calibration and therefore directly impacts the reliability of the sample measurement.

How long pH buffers last when opened?

Following the manufacturer's recommendation a bottle with pH buffer should only be opened for taking out a small volume for calibration and must be closed quickly afterwards.

pH buffer solutions in open beakers for calibration should not be used longer than 10-15 minutes (pH 4 and 7). Alkaline buffers (pH 10 or 12) are very sensitive to CO_2 from ambient air and will quickly change their pH. Those should be used no longer than 5-10 minutes, depending on the pH probe stabilisation time and temperature.

At lower temperatures (0-20 °C) pH buffers are more stable than at higher temperatures (20-40 °C). Above 40 °C pH buffers (and samples) should be measured in a closed vessel with cover. Otherwise too much water can vaporise, changing the concentration of the buffer or sample and therefore the pH value. In addition the equilibrium between solution and steam/air phase can change the buffer pH as well.

Reagents

1. Deionised water.
2. Potassium chloride solid (Analar or equivalent grade).
3. Buffer solutions, pH 4.00, 7.00
4. Trigene/Distel® disinfectant.

Procedure

1. Weigh 10 g (<2mm, air dry, well mixed sample) into a clean 40 mL container.
2. Using a measuring cylinder add 25 mL of deionised water.
3. Shake the samples for 1 hour using a reciprocating shaker at 150 rpm.
4. Allow to stand for 1 hour.
5. Calibrate pH meter using fresh pH 4 and 7 buffers.
6. Measure the buffers to check the calibration, if the measured values deviate ± 0.01 pH units from the actual buffer value repeat the calibration, replace the buffers if necessary.
7. After 1 hour - if the solution is clear immerse pH meter electrode the liquid and measure the pH.

   After 1 hour - if the solution is not clear, add a stirrer bead to the sample and place the container on a magnetic stirrer.
8. Record the pH when the reading has stabilised. Report the result as pH-H_2O.

   Note: the reading is considered stable when it does not change more than 0.1 unit per 30 seconds (or 0.02 units per 5 seconds). In calcareous soils stabilisation may be difficult to achieve because of non-equilibrium conditions.
9. To prevent between-sample contamination rinse electrode with deionised water.
10. After every 10 samples measure a pH buffer to check for instrument drift. If the pH of the buffer deviates more than ± 0.01 pH units recalibrate the pH meter.
11. After measuring pH-H_2O add 1.86 g KCl (1M) to the sample and shake on reciprocal shaker for 1 minute.
12. Allow to stand for 10 minutes.
13. Measure the pH, whilst stirring, record the result when the reading has stabilised. Report as pH-KCl.

Reporting of results

Report as pH (H_2O) 1:2.5 soil: solution and pH (KCl 1.2.5 soil: solution). Replicated pH measurements typically show close agreement, it the difference between replicates is $\pm$ 5 % repeat the analysis before reporting.

3.3 Cations using Silver-Thiourea and determination of Cation Exchange capacity (CEC)

a. Scope

A single extraction method using silver-thiourea for measuring exchangeable cations and effective CEC in soils with variable charges. In this procedure soil is extracted using a mixture of silver nitrate (0.01 M) and thiourea (0.2 M) following Pleysier and Juo, 1979, variations of the method exist using 0.1 M thiourea.

b. Quality Control

The samples should be analysed in random order rather than analysis by sample collection order/pit/plot etc. Analyse a sample replicate every 10 samples and two blank samples per batch. Although there is no available reference material for this determination, it is recommended that DC85104 (Available nutrients GBW07415) be used. This is certified for some exchangeable bases, and will be used as an 'in-house' reference material for other elements.

d. Equipment

- Reciprocal shaker
- 50 ml centrifuge tubes
- Centrifuge
- 1 litre clear volumetric flask – class A
- 500 ml volumetric flask – class A
- 2.5 L amber bottle equipped with reagent dispenser
- 2 litre volumetric flask – class A
- Magnetic stirrer and stirrer bead
- Sieve strainers
- Soil certified reference material (CRM): NSCDC85101a Available nutrients in soil

ICP equipment (analyst only)

- Argon humidifier
- Baffled spray chamber
- High solids concentric nebuliser (C2 Aerosalt)

e. Reagents

Soak glassware in 0.5 M nitric acid followed by rinsing three times with deionised water.

Extraction reagents – prepare all reagents on the day of extraction

1. Silver nitrate solid (Certified grade or equivalent grade)
2. Thiourea solid
3. Concentrated nitric acid (Certified or equivalent grade)
4. Silver nitrate, 0.02 M: half fill a 1 L volumetric flask with deionised water, add 3.3974 g of silver nitrate, dilute to volume stopper the flask and shake to mix.
5. Thiourea solution, 0.2 M: half fill a 500 mL volumetric flask with deionised water, add 30 g of thiourea, dilute to volume, stopper the flask and shake to mix.

6. Silver thiourea reagent, pH 5.5: decant the thiourea solution made in step 5 into an amber glass bottle, equipped with a reagent dispenser, add a magnetic stirrer bead to the bottle, turn on the magnetic stirrer and ensure the bead is spinning. Slowly add 1 L of 0.02 M silver nitrate prepared in step 4. Continue to stir whilst adding another 500 mL of deionised water, to prepare 2 L of reagent. **Warning do not reverse this order.**

Silver nitrate, 0.02 M		Thiourea 0.2 M	Final volume, mL	Extracts n number of samples
Weight, g	Volume, mL	Weight, g		
3.3974	1000	30	2000	66
1.6987	500	15	1000	30
0.8494	250	7.5	500	15

Analysis only

7. Nitric acid for standard preparation (for trace metal analysis)
8. Nitric acid for acid washing (Certified grade or equivalent)
9. ICP matrix solution (ICP analyst only) acidified thiourea: half fill a 500 mL volumetric with deionised water, add 7.5 g of thiourea, add 50 mL concentrated acid, dilute to volume with deionised water, stopper the flask and shake to mix.
10. LAST RESORT - DILUTES THE SAMPLE. Internal standard, 1ppm yttrium, scandium, gallium and scandium: half fill a 2 L volumetric flask with deionised water, add 2 ml of each internal standard listed, dilute to volume with deionised water, stopper the flask and shake to mix. Check for interferences before deciding which lines to choose for the internal standards (normally use as a matter of course yttrium 371.029 and scandium 424.682). Ensure that, when post-processing, the appropriate internal standard is used for each element.
11. Stock standards, commercially prepared ICP-OES single element standards 10,000 ppm diluted in nitric acid: Al, B, Ca, Co, Co, Fe, K, Mg, Na, Mn, Mo, Ni, P, Zn.
12. Intermediate standard 1 containing all 14 elements – 100 mg/L: half fill a 25 mL volumetric flask with deionised water, add 250 µL of each of the 10,000 ppm standards (Al, B, Ca, Co, Co, Fe, K, Mg, Na, Mn, Mo, Ni, P, Zn), dilute to volume, stopper the flask and shake to mix.
13. Intermediate standard 2 containing 6 elements – 1000 mg/L: half fill a 25 mL volumetric flask with deionised water, add 2500 µL of each of the 10,000 ppm standards (Al, Ca, Fe, K, Mg, Mn)
14. Calibration standards: prepare from intermediate standard 1 and 2 in acidified thiourea, see table 1.
15. ICP multi-element standard VIII 24 elements in dilute nitric acid 100 mg/L, commercially prepared.
16. ICP multi-element standard IV 22 elements in dilute nitric acid 1000 mg/L, commercially prepared.

17. Suitable water CRM: pipette 5 mL of CRM into a 15 mL tube, add 5 mL acidified thiourea. Cap and shake to mix.
18. Suitable soil certified reference material (CRM) with known amounts of the elements of interest (please use NCSDC85101A or available equivalent)
19. VKI WW1B for phosphorus: half fill a 10 mL volumetric flask with acidified thiourea, add 100 µL of VKI WW1B, dilute to volume, stopper the flask and shake to mix.

Table 1 Calibration standards

Standard	ppm	Volume, mL	Amount to pipette, µL	Stock standard, ppm	Elements
1	0.25	50	125	100	All
2	0.50	50	250	100	All
3	1.0	50	500	100	All
4	2.5	50	1250	100	All
5	5.0	50	2500	100	All
6	10	50	5000	100	All
7	25	25	625	1000	Al, Ca, Fe, K, Mg, Mn
8	50	25	1250	1000	Al, Ca, Fe, K, Mg, Mn
9	75	25	1875	1000	Al, Ca, Fe, K, Mg, Mn
10	100	25	2500	1000	Al, Ca, Fe, K, Mg, Mn

f. Procedure

1. Weigh 5 g (<500 µm, air dry soil, well mixed) sample into a 50 mL centrifuge tube, record the actual weight.
2. Add 30 mL of silver thiourea to each sample, blank (x3) and CRM (x2).
3. Shake for 2 hours on a reciprocal shaker.
4. Centrifuge at 2000 rpm for 15 minutes. If the supernatant isn't clear after 15 minutes repeat for a further 15 minutes.
5. Using a dropping pipette if necessary, transfer a minimum of 20 mL of particulate free supernatant to a clean 50 mL centrifuge tube.
6. If organic matter/ particulates remains in the supernatant pour the sample through a cell strainer into the 50 mL centrifuge tube.
7. The particulate free extract is ready for analysis by ICP, as per manufacturer's instructions, for Ca, Mg, K, Na and Al (plus other metals if requested).
8. Extract reagent blanks, suitable soil CRM and 10 % replicates per batch of samples, label replicates with '*' to denote that the sample is a replicate sample. Record the name of the CRM used on the tube presented to the analyst.

g. Disposal

Transfer all solutions for disposal by a chemical waste company.

h. Calculating CEC

Convert from volume concentration (mg/l) measured by ICP OES to mass concentration (mg/kg):

$$\text{mg kg}^{-1} = \frac{\text{(mg L}^{-1}\text{ for sample – mg L}^{-1}\text{ for blank) x final volume, mL}}{\text{Sample weight, g}}$$

Example for K:

Concentration mg/l 8.454
Concentration in blank 0.117
Volume 30 ml
Sample mass 5g
((8.454 – 0.117) * 30) / 5 = 50 mg/kg

Divide cation molar mass by its valence:

The valence of an element is a measure of its combining power with other atoms when it forms chemical compounds or molecules

Al^{3+} (molar mass 26.98g) 1 mole positive charge 26.98 g/3 = 8.99g

Ca^{2+} (molar mass 40.08g) 1 mole positive charge 40.08 g/2 = 20.04g

K^{+} (molar mass 39.10g) 1 mole positive charge 39.10 g/1 = 39.10g

Mg^{2+} (molar mass 24.30g) 1 mole positive charge 24.30 g/2 = 12.15g

Na^{+} (molar mass 23.00g) 1 mole positive charge 23 g/1 = 23.00

Convert to mmols equivalent (mg/kg to mmol/kg): .

mmol/kg = ((conc mg/kg * 1mole positive charge) / (molar mass / valence)) * (1 $cmol^{+}$ /10$mmol^{+}$) * 10

(In excel)

Example for K:

Soil concentration of K^{+} = **50 mg/kg**:

1 mole of + charge of K is 39.10 g

1 $cmol^{+}$ = 1 mole/100 of positive charge

1 $mmol^{+}$ of positive charge = 39.10 mg of K

((50mg/kg * 1 / (39.10/ 1)) * 0.1) * 10 = 1.3 $mmol^{+}$/kg

Example for CA:

Soil concentration of Ca^{+2} = **50 mg/kg**:

1 mole of + charge of Ca^{+} is 40.08 g / 2 = 20.04 g

1 $cmol^{+}$ = 1 mole/100 of positive charge

1 $mmol^{+}$ of positive charge = 20.04 mg of K

((50mg/kg * 1 / (40.08/ 2)) * 0.1) * 10 = 2.5 $mmol^{+}$/kg

Simplified: Divide concentration mg/kg by mmol positive charge =

K 50 mg/kg = 50/ 39.10 = 1.3

Ca 50 mg/kg = 50/20.04 = 2.5

Al 50 mg/kg = 50/8.99 = 5.6

Mg 50 mg/kg = 50/12.15 = 4.1

Na 50 mg/kg = 50/23 = 2.2

Sum cations: (**Exchangeable Cation Exchange Capacity (eCEC))**

eCEC $mmol^{+}$/kg = Σ Na + K + Mg + Ca + Al

3.4 Particle Size by Gravimetry – Soil Texture

a. Scope

Separation of the mineral part of the soil into various soil fractions and determination of the proportion of these fractions.

b. Equipment

- 1 L Schott bottle
- 1 L measuring cylinder
- Large weighing boat
- Analytical balance
- 250 mL conical flasks
- Spatula
- 100 mL measuring cylinder
- 25ml measuring cylinder
- Watch glasses
- Blender (milk shake maker)
- 53 µm sieve (50 µm not available to purchase)
- Wide neck bowl with handle and pouring spout
- 1L plastic beaker for waste
- Glass sedimentation cylinder marked at 1 L
- Thermometer
- Plunger
- Stopwatch
- 20 mL pipette and pipette filler
- Wash bottle
- Evaporating basins
- Disposable tin evaporating basins
- 1g weight
- Metal tray
- Drying oven
- Desiccator
- Autoclave (for disposal of non-EU soil/water waste)
- 12 x 1 L autoclaveable bottles

c. Quality Control

The samples should be analysed in random order rather than analysis by sample collection order/pit/plot etc. Wash any containers that are to be used in the gravimetric procedure with deionised water. Ignite evaporating basins at 500°C to remove remaining residue. When the items are clean and dry store in a clean dust free environment (desiccator). Ensure the balance is level on the work surface in a draught/vibration free environment. Check the balance using a calibrated mass before and after use.

d. Reagents

1. Calcium rich soil – 5 % sodium hexametaphosphate (aka Calgon): add 50 g of sodium hexametaphosphate to 1000 mL of deionised water. Shake well to mix. A mechanical shaker can be used to aid dissolution.
2. Oxisol soils – 6 % NaOH: add 60 g of sodium hydroxide to 1000 mL of deionised water.

e. Procedure

a. Weigh 10g (<2mm, air dry, well mixed) sample into 250ml conical flask, record the sample weight **(W_1).**

b. Using a measuring cylinder add 100ml of deionised water.

c. Using a measuring cylinder add 20ml of dispersing agent (sodium hexametaphosphate or sodium hydroxide)

d. Swirl to mix. Cover the conical flask with a watch glass.

e. Allow to stand overnight.

f. With washings transfer the contents of the conical flask to a blender beaker.

g. Mix, using a blender, for 10 minutes.

h. Pass sample through 53μm sieve, retain the sand fraction on the sieve and collect clay/silt fraction in a receiver (large washing up bowl).

i. Use a wash thoroughly bottle to wash the sand fraction, retaining the liquid in the wide neck bowl.

Sand (50 – 2000μm)

j. Transfer the sample retained on the sieve to a pre-weighed evaporating tray, using de-ionised water bottle to wash/move the sand into the tray.

k. Oven dry for two days at 105°C.

l. Allow to cool in a desiccator, reweigh the sample (**W_2**).

Clay (<2μm)

a. Decant the remainder of sample from the receiver into a 1 litre sedimentation cylinder and dilute to the mark with deionised water

b. Use a plunger to mix the sample thoroughly (20x)

c. Measure the temperature of the solution in the sedimentaion cylinder

d. After the amount of time specified in the table below pipette 20ml from 5cm below the surface of the liquid

e. Transfer the contents of the pipette to a pre-weighed tin cap

f. Oven dry at 105°C for two days

g. Allow the tin cap to cool in a desiccator and reweigh **(W_3)**

Silt (2 – 50μm)

h. Silt is obtained by subtraction, see calculations

Include a blank sample with each batch of samples: conical flask with water from the same source plus dispersing agent) for temperature correction in the clay determination and for correction of the dispersing agent addition. Follow steps b – g and I – r. The blank value **(W_4)** is used in the calculation.

Temperature (°C)	Time
18	4 hr 12 min
19	4 hr 6 min
20	4 hr 0 min
21	3 hr 54 min
22	3 hr 48 min
23	3 hr 43 min
24	3 hr 38 min
25	3 hr 33 min

f. Calculations

Clay (<2μm) = (**W_3** x 50) – (**W_4** x 50) wt **A**

Sand (>50μm) = **W_2** wt **B**

Silt (2 – 50μm) = **W_1** – (**A** + **B**) wt **C**

Sample weight **Z** = **A** + **B** + **C**

The proportional amounts of the fractions can now be calculated by:

% Clay (<2μm) = **A**/sample weight **Z** x 100

% Silt (2 – 50μm) = **C**/sample weight **Z** x 100

% Sand (> 50μm) = **B**/sample weight **Z** x 100

g. Housekeeping

Sample disposal: autoclave soil/liquid waste 121 °C must be maintained in the centre of the load for 30 minutes.

Wash all equipment and return to original storage location. When you have completed the analysis and checked the data dispose of all your samples and extracts. Unlabelled samples will be thrown away.

Soak all glassware in dilute Decon 90 (wear gloves) use a green scourer to remove pen marks, use a bottle brush to clean the insides to remove as much residue as possible before transferring to the dishwasher. Dispose of single use plastic.

3.5 Carbon and Nitrogen by Combustion

Introduction

In this procedure finely homogenized soil, sediment or plant is weighed into a tin capsule before being introduced into an elemental (combustion analyser) to capable of determining the concentrations of Carbon (C) and Nitrogen (N). The analyser is routinely configured to measure carbon and nitrogen. The amount of sample weighed and the capsule size used depends on the sample type, expected levels of C and N sample homogeneity.

Measuring principle: carbon and nitrogen.

The samples are weighed in tin or silver vessels and loaded in the carousel for 120 samples. The C and N elemental analysis is based on the high temperature combustion and subsequent analysis of the combustion gases. The quantitative separation of the analyte gases N_2, CO_2, H_2O and SO_2 in the He carrier gas prior to the detection is crucial for the performance of the complete instrument. All four analyte gases are separated on one column, by the use of 4 stepped temperatures. At near room temperature, N_2 flows unimpeded through the column while CO_2, H_2O and SO_2 are adsorbed. After the detection of the N_2 peak, the column temperature is quickly increased to approx. 60 °C, which releases the CO_2. After detection of the CO_2, the column is stepped to over 100 °C for release and detection of the H_2O. Finally, the SO_2 is desorbed at a column temperature of over 200 °C.

Equipment

Oven capable of achieving 40 °C

- Mixer mill or cutting mill
- 6 figure analytical balance
- Calibration mass 20 mg
- Tin capsules 5 x 5.5 mm
- 96 position tray
- Tweezers and micro spatula
- Metal block
- Appropriate certified reference material

Reagents

- Sulphanilic acid (C, H, N & S)
- Polyethylene (oxygen)
- Benzoic acid (oxygen)

Procedure

1. Air or oven dry samples at 40 °C.
2. Grind samples to <100 µm using a mixer mill.
3. Check the balance is clean and level.
4. Check the performance of the balance before, periodically and at the end of sample weighing.
5. Place a tin capsule on the 6 figure balance and press tare.
6. Remove the tin capsule from the balance and place on the metal block.
7. Carefully transfer soil into the tin capsule using the microspatula.
8. Pick the capsule up with tweezers and tap the tweezer with the microspatula to dislodge any material adhering to the base of the capsule.

9. Return the capsule to the balance to determine if the desired weight has been obtained or more sample needs to be added/removed.
10. One the required amount of sample has been obtained use the tweezers to fold the parcel into a cube.
11. Weigh and record the weight of the prepared sample in the sample weighing sheet.
12. Transfer the prepared sample to a clean/residue free 96 position tray in the well that corresponds to the weighing sheet.
13. Repeat for each sample.
14. Include an appropriate CRM at the start and end of the sample set.
15. In each batch of samples include 10 % duplicates at the end of the sample set.
16. Weigh sulphanilic acid for calibration and drift standards.
17. The samples are now ready for analysis of carbon and nitrogen following manufacturer's instructions.

Typical weights for samples, calibration standards and CRMs for C, H, N & S

Type	**Organic soil**	**Mineral soil**
Bypass	Tin capsule	Tin capsule
Cal std 1	0.4	0.1
Cal std 2	0.8	0.2
Cal std 3	1.6	0.4
Cal std 4	2.4	0.6
Cal std 5	3.6	0.8
Cal std 6	4.0	1.0
Drift	1.5	0.25
Drift	3.0	0.5
CRM	B2150 & B2152 6	ES4 & B2152 8
Typical sample	6	8

Analyser

Elementar vario MICRO cube

Certified Reference material values

CRM	**Carbon (C) %**	**Nitrogen (N) %**
IRMM443-4 (ES4 Eurosoil 4)	1.45	0.16
B2147 Sulphanilic acid	41.71	8.00
B2152 Low organic soil	1.65	0.14

SO-1 Canmet soil	0.25	0.04
SO-2 Canmet soil	4.8	0.22
SO-3 Canmet soil	6.6	0.02
SO-4 Canmet soil	4.4	0.04
Humic acid powder*	35.90	0.86
NJV943 Energy forest (Salix)	_	0.37
SQC014 Nutrients in soil	_	_

Check the CRM certificate for uncertainty values.

Values in brackets are not certified – for information only

3.6 Total Phosphorus

a. Introduction

It is expected that this procedure will take approximately 6 hours of contact time spread over multiple days. The digestion block is capable of digesting 40 tubes at a time, of those some spaces will be used for blanks, certified reference materials and replicate samples, expect to extract 32 samples per batch. The method is taken from the final stage of step from 'Characterisation of available P by sequential extraction'.

b. Equipment

- Analytical balance
- Hot block digester with manifold
- 100 mL digestion tubes
- 100 mL measuring cylinder
- Spatula
- Deionised water wash bottle
- 5 mL pipette and pipette tips
- Acid reagent dispenser
- Vortex mixer
- 50 mL centrifuge tubes (Sarstedt clear cap)
- Anti-bumping granules
- Ceramic mats
- Bottle brush
- Acid bath

c. Extraction reagents

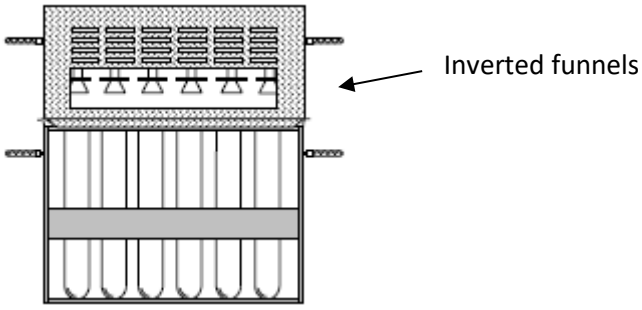
- Hydrogen peroxide (H_2O_2) Primar grade 30 % (100 volumes)
- Sulphuric acid (H_2SO_4) analytical grade
- Suitable soil certified reference material – CANMET SO-3

Glassware cleaning reagents

- 37 % hydrochloric acid commercially prepared (analytical grade)
- 10 % hydrochloric acid – half fill a 5 L volumetric flask with deionised water, slowly and carefully add 1350 mL of 37 % concentrated hydrochloric acid, dilute to volume, stopper the flask and shake to mix. Carefully decant into an acid bath.
- Decon^{TM} Contrad 70 liquid detergent

Glassware cleaning procedure

1. Wash dirty tubes in the sink with a dilute solution of Decon^{TM} Contrad 70 liquid detergent diluted in hot water. Scrub the insides clean with a bottle brush and rinse well with tap water. Remove pen marks with the aid of a green scourer.
2. Soak the tubes overnight in 10 % HCl. Rinse three times with type 2 deionised water and one final rinse of type 1 deionised water. Allow to dry naturally or oven dry. Replace the acid when it has been used five times, sooner if dirty.
3. Remove the manifold and rinse the inverted glass funnels, drench thoroughly with tap water followed by thorough rinsing with deionised water. Allow to dry before re-use.



d. Procedure

4. Weigh 0.5g +/- 0.1g (<2mm soil) into directly into a glass tube (stand the tube in a measuring cylinder on the balance) record the actual weight, label the tube twice to prevent sample IDs being lost and keep the samples in rack order in the event that sample IDs are lost,
5. Using an automatic pipette Add 5 mL of deionised water.
6. Allow to stand overnight,
7. Using a reagent dispenser add 5 mL of sulphuric acid and mix using a vortex mixer.
8. Add 1 anti-bumping granule.
9. Heat the samples using a hot block at 110°C for 30 mins, then increase the temperature slowly every 30 minutes to evaporate the water at the following temperatures: 125°C, 150°C, 175°C, 200°C, 225°C, 250°C, 275°C, 300°C and 360°C
10. Remove from the rack of tubes from the hot block after the final heating of 360°C and allow the tubes cool for 30 minutes. Maintain the temperature of the hot block at 360°C.
11. Once the tubes are cool (to hand warm) add 0.5cm^3 of H_2O_2, vortex mix and return to the hot block to heat for 30 minutes.
12. Repeat step 11 about an additional nine times until your solution is clear then the reaction is complete.
13. On the 10th addition of H_2O_2 once your solution is clear return the tubes to the block and heat for 60 minutes to remove any remaining H_2O_2. Residual H_2O_2 interferes with the phosphorus determination.
14. Remove the samples from the rack and allow to cool for 30mins.
15. Vortex mix the sample thoroughly to ensure the residue remaining in the digestion tube is fully broken down and in solution: carefully hold the digestion tube at an angle against the side of the vortex mixer rubber cup to tap the tube gently against the body of the vortex mixer. Then place the digestion tube onto the rubber cup to mix for 20 seconds.
16. Transfer with washings to a 50 mL centrifuge tube and dilute to 50mL with deionised water. Stopper the tube and shake well to mix.
17. Allow residue to stand overnight at room temperature to permit silica to settle out.
18. The sample is now ready for analysis by continuous flow auto-analyser.
19. Extract reagent blanks, suitable soil CRM and 10 % replicates per batch of samples, label replicates with '*' to denote that the sample is a replicate sample.

20. Follow the cleaning procedure for the glass tubes and inverted funnels on the digestion block.

    NB if the samples have been refrigerated allow the samples to equilibrate to room temperature after removal from the fridge – failure to do this will result in over-estimation due to inaccurate dilutions.

    Timeline:
    - Day 1 weigh samples and add deionised water.
    - Day 2 add acid and apply heat (program 1).
    - Day 3 continue heating (program 2) and begin additions of H_2O_2.
    - Day 4 continue additions of H_2O_2 and complete the final digestion at 360 °C.
    - Day 5 dilute to volume and clean digestion tubes.

Calculations

$$\text{mg kg}^{-1} = \frac{(\text{mg L}^{-1} \text{ for sample} - \text{mg L}^{-1} \text{ for blank}) \times \text{final volume, mL}}{\text{Sample weight, g}}$$

Analyser

Skalar San ++ Continuous flow autoanalyser.

3.7 Bulk Density (BD) Field samples

Field procedure

1. Samples should be collected vertically (preferred) or horizontally from the face of a soil pit.
2. Measure and record the height (h) and diameter (d) of the bulk density rings in cm.
3. Use a trowel or knife to prepare the soil surface, clearing away any loose stones or debris.
4. Place the bevelled edge of the cylinder against the soil, and place the driving tool (piece of wood) against the other end of the cylinder.
5. Gently hammer the cylinder into the soil until the soil projects 3 mm out of the cylinder. Try to keep the cylinder at right angles to the soil face (i.e. horizontal if sampling a vertical face).
6. Gently excavate the cylinder plus soil using a knife and trowel, leaving extra soil extending from each end of the cylinder.
7. Carefully trim the soil flush to the ends of the cylinder and put on caps. The aim is collect a full cylinder of soil i.e. try not to lose any soil from the cylinder.
8. Place the samples in a small self-seal polythene bag.
9. Clearly label the bag.
10. Return to the lab to process the samples as soon as possible.

Lab Procedure

1. Label and weigh small foil tins (label the tins by etching the sample ID on the underside of the tin). Record the sample number and weight of empty tin [**W1]**.
2. Push out the soil from the metal rings into the tins. You may need to use a spatula to aid removal of soil from the rings.
3. Place the samples on a metal tray and allow to oven-dry to constant weight at 105 ^{o}C overnight.
4. Remove the samples from the oven and place in a desiccator to cool.
5. Reweigh the oven dry soil plus foil tin. Record the weight [**W2].**
6. Calculate bulk density in g cm^{-1}.

Note

Constant weight is achieved when of the weight of the sample remains the same after additional cycles of heating, cooling and re-weighing. Usually leaving the samples overnight is adequate to avoid having to repeat the cycle. For precise work check that constant mass has been achieved, by repeating steps 3 to 5 as many times as necessary.

Calculation

Volume of soil (cm^3) = $\pi r^2 h$

Where r= radius of ring and h=height of ring, both in cm.

$$\text{Bulk Density (g cm}^{-3}) = \frac{\text{Weight of oven dry soil [W2 – W1] (g)}}{\text{Volume of soil (cm}^3)}$$

3.8 Bulk Density from Loss of Ignition (LOI)

For the studies where soil bulk densities are not available, the densities are estimated as follows

$$BD = \frac{100}{\frac{\%OM}{0.244} + \frac{100 - \%OM}{1.64}}$$

= (100)/ ((%LOI/0.244) + ((100-%LOI)/1.64))

%OM = %LOI

3.9 Loss on Ignition

Apparatus

Oven capable of maintaining a temperature of 150 ºC ± 2.5 ºC
Balance, readable to 1 g
Balance, readable to 0.001 g
Desiccator, containing anhydrous silica gel
Crucibles
Electric muffle furnace capable of maintaining a temperature of 550 ºC ± 25 ºC

Procedure

Preparation of crucible

Before carrying out determinations, carry out a test on the empty crucible

1. Place the crucible in the muffle furnace, heat to 440 ºC ± 25 ºC and maintain temperature for 1 hour.
2. Remove the crucible from the furnace and allow it to cool to room temperature in the desiccator.
3. Weigh the crucible to the nearest 0.001 g (mc)

Preparation of test sample

1. Place each sample in a prepared crucible and dry in the oven at a temperature of 105 ºC ± 2.5 ºC. The sample is deemed dry when the difference in successive weighing's, carried out at intervals of 4 hours, do not exceed 0.1 % of the original mass of the sample.
2. Allow the samples to cool to room temperature in the desiccator and weigh each crucible to 0.001 g (m3).

Ignition of soil

1. Place the crucible in the unheated muffle furnace, heat to 550 ºC ± 25 ºC, and maintain this temperature for a period of not less than 3 hours or until constant mass is achieved.
2. Remove the crucible from the furnace and allow to room temperature in the desiccator.
3. Weigh the crucible and contents to the nearest 0.001 g (m4).

Calculations

Calculate the percentage of the original soil sample passing the 2 mm test sieve from the equation:

$$\text{Fraction finer than 2 mm} = \frac{m2}{m1} \times 100$$

Where m_1 is the original mass of dry sample (in g);

m2 is the mass of sample passing the 2 mm sieve (in g)

Calculate the mass loss on ignition, LOI as a percentage of the dry mass of soil passing a 2 mm test sieve from the equation

$$LOI = \frac{m3 - m4}{m3 - mc} \times 100$$

Where m3 is the mass of the crucible and oven dry soil specimen (in g);

m4 is the mass of the crucible and specimen after ignition (in g);

mc is the mass of the crucible (in g)

4. School of Geography, University of Leeds Laboratory Analysis Detection Limits

Overview:

The detection limit of an analytical method is the lowest quantity of a substance that can be distinguished from the absence of that substance (a *blank value*) with a stated confidence level. For example, when determining the concentrations of a specific metal/nutrient within a soil sample, what is the lowest level of concentration (mg/kg) that can be detected using a specific method to extract metals/nutrients from the soil and the type of instrument used to determine these concentrations.

There are a number of different types of detection limit commonly used. These include the **Instrument Detection Limit** (**IDL**), the **Method Detection Limit** (**MDL**) and the **Practical Quantitation Limit** (**PQL**)

Detection limits can be estimated in different ways: from the mean of the blank, the standard deviation of the blank, the slope (analytical sensitivity) of the calibration plot and a defined confidence factor.
At the UoL the IDL and MDL are first determined and whichever is the highest value is then used as the PQL. The PQL is the value used for minimum value that the database will accept as an acceptable result.

Definitions:
IDL: expressed as mg/l, calculated by multiplying threefold the value of the standard deviation of ten measurements of blank solution used for instrument calibration;

MDL: expressed as mg/kg, calculated by multiplying tenfold the value of the standard deviation of measurements of six solutions of analytical blanks prepared on different working days.

PQL: this is the lowest level at which an analytical method can confidently discern between two different values. This is the level at which we feel confident reporting results or the level at which uncertainty becomes unacceptable.

Reagent blank: the blank sample is a sample taken through the same experimental conditions as employed in the actual analysis, but omitting the sample. The purpose of the reagent blank is to determine the amount of impurities introduced from chemicals and equipment used in the procedure.

Analyte: a chemical substance that is the subject of chemical analysis

Calibration graph: is determined by measuring the concentration of solutions containing known amounts of the analyte of interest, and then constructing a graph in which the measured response is plotted against instrument response. The concentration of an unknown solution can therefore be determined from the calibration graph.

Standard: a solution containing a known concentration of an element or a substance. A known mass of element/substance is dissolved to make a specific volume. Standard solutions are used to calibrate instruments.

5. Soil Analysis Type, Units of measurement and max min and detection limits values in the database

Soil Analysis Type ID	SoilAnalysisTypeName	Unit Of Measure	Minimum Value	Maximum Value	PQL
1	Roots	%	0	20	
2	Stones	%	0	90	
3	Soil	%	10	100	
4	Sand	%	0	100	
5	Silt	%	0	100	
6	Clay	%	0	100	
7	pH (H2O)		2	8.5	
8	pH (KCl)		2	8.5	
9	Bulk Density	g cm-3	0.2	4	
10	Bulk Density from LOI	g cm-3	0.2	4	
11	LOI	%	0	100	
12	Nitrogen	%	0	5	
14	Carbon	%	0	80	
15	Available Phosphorous	mg/kg	0	200	
16	Total Phosphorous	mg/kgs	0	2000	
17	Total Phosphorous 0-30cm composite sample	mg/kgs	0	2000	
18	Al	mg/kg	0	2100	
19	Ca	mg/kg	0	13000	
20	K	mg/kg	0	1500	
21	Mg	mg/kg	0	900	
22	Na	mg/kg	0	400	
23	B	mg/kg	0	10	
24	Ba	mg/kg	0	150	
25	Co	mg/kg	0	20	
26	Cr	mg/kg	0	10	
27	Cu	mg/kg	0	20	
28	Fe	mg/kg	0	300	
29	Mn	mg/kg	0	1500	
30	Mo	mg/kg	0	150	

31	Ni	mg/kg	0	10
33	Se	mg/kg	0	10
34	Si	mg/kg	0	150
35	Sr	mg/kg	0	60
36	V	mg/kg	0	10
37	Zn	mg/kg	0	10
38	S	mg/kg	0	10
39	Ti	mg/kg	0	10
40	eCEC	mmol/kg	0.5	150
41	CEC pH7	mmol+/kg	0	2000
42	Al Total Digestion	mg/kg	0	2100
43	Ca Total Digestion	mg/kg	0	13000
44	K Total Digestion	mg/kg	0	1500
45	Mg Total Digestion	mg/kg	0	900
46	Na Total Digestion	mg/kg	0	400
47	Fe Total Digestion	mg/kg	0	300

6. Lab Analysis Method References

Sample Preparation: - sieving, weighing and subsampling: - modified from **ISRIC**: Technical paper 9, sixth edition, **and pp.1-1**.

pH: - modified from **ISRIC pp.4-1.**

Bulk Density: - D.L. Rowell, Soil Science, Methods and Applications, Ch.4, pp69 – 78. Longman Group UK 1994.

Bulk Density from Loss on Ignition: - Post WM*,* Kwon KC *(*2000*)* Soil carbon sequestration and land-use change: processes and potential*.* Global Change Biology*,* 6*,* 317–327*.*

Particle Size: Adapted from Procedures for Soil Analysis, L.P.van Reeuwijk, International Soil Reference and Information Centre, Sixth Edition, 2002.

CN: - manufactures recommendations for ground fine material using a Gas Combustion Analyser, Elementar Vario EL Cube.

CEC:- Plesier, J.L. and Juo, A.S.R., 1980. A single-extraction method using silver-thiourea for measuring exchangeable cations and effective CEC in soils with variable charges. ***Soil Science****.* Vol. **12**, No. 4. 205 – 211

Marco Grotti, Emanuele Magi and Riccardo Leardi, 2003. Selection of internal standards in inductively coupled plasma atomic emission spectrometry by principal component analysis. ***J. Anal. At. Spectrom.***, Vol. **18**, 274-281

Measured with an **ICP_OES** Pella, E.1990. Elemental organic analysis. Part 1, Historical developments. *American Laboratory* 22 (2):116–25.

Loss on Ignition: - Allen SE. Chemical Analysis of Ecological Materials, 2nd Edition, CH2, pp.15. Blackwell, 1989.

Phosphorous: - modified (final steps) from: Tiessen H. and Moir J.O. 'Characterisation of available P by sequential extraction', University of Saskatoon, Saskatchewan, Canada.

7. References

Elementar: Gas Combustion Analyser, Elementar, Vario EL Cube Analyser for CHNS-O determination in Solid and Liquid samples, Instruction Manual**.**

ICP-OES: Thermo Fisher Scientific, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) – iCAP7600 Duo.

ISRIC: 'L.P. van Reeuwijk, Procedures for Soil Analysis, technical paper 9, sixth edition, International Soil Reference and Information Centre' ISRIC 2002.

MAFF/ADAS: The Analysis of Agricultural Materials, Ministry of Agricultural fisheries and Food (MAFF), reference Book 427, Third edition 1987. ISBN 0 11 242762 6.

8. Appendix

8.1 RAINFOR (Normal) Field Soil Sampling Protocol

Soil Pit:

Choose a pit location, outside of the plot, that represents best the topography of the plot and with the same vegetation as the plot. For example, if the plot is on a slope with a prominent aspect of south west, then locate the pit along X or Y, whichever slopes south west and has more or less a representative plot gradient and vegetation (this is not that easy). Dig a pit 2m deep 2x1m square.

Record Pit Site details:

- Plot name
- Plot code
- Date/time (local)
- Name of sampler
- Location (GPS and in relation to plot, example: 'opposite subplot 22, 1m west of line, nearest tree 231')
- Describe area around plot: vegetation, topography, anthropogenic disturbance, aspect, slope, is the area around the pit stony or are there boulders present, termite mounds, when did it last rain and for how long; plus any other information that may be relevant.

Soil Profile description:

- Delineate horizons
- Record horizon depths
- Describe soil colour, motteling, texture, structure, moisture, consistency, density of each horizon.
- Describe pit drainage, root abundance and horizon boundaries.

Pit Soil Sampling:

- Take one sample, for nutrients analysis in the labs, at the following depths (cm) down the profile:
  0-5,
  5-10,
  10-20,
  20-30,
  30-50,
  50-100,
  100-150,
  150-200,
- From the bottom of the pit, use the corer to extract further nutrient samples at the following depths(cm):
  200-250
  250-300
  300-350
  350-400

Note: this may not always be possible, but we must assume that it is!

The bag samples are labelled:

- Date
- Plot code
- Pit nutrients
- Depth
- Country

Separate samples are taken for Bulk density measurement only and are taken at the same depths as the nutrient samples.

Fill the pit bag in!

The Holes/Cores

Five holes are cored at random locations (subplots) from within the plot; Record hole site/subplot details:

- Plot name
- Plot code
- Subplot number
- Date/time (local)
- Name of sampler
- Location: example 'subplot 15, nearest tree 94')
- Describe subplot: vegetation, topography, anthropogenic disturbance, aspect, slope, is the area around the pit stony or are there boulders present, termite mounds, when did it last rain and for how long; plus any other information that may be relevant (i.e. tree fall, gap in canopy)

Hole (Core) Soil Sampling:

- With a corer take samples at the following depths (cm):
  0-5,
  5-10,
  10-20,
  20-30,
  30-50,
  50-100,
  100-150,
  150-200,

Note: It may not always be possible to reach 200cm, whatever depth can be reached is recorded in the field note book and on the sample bag.

The bag samples are labelled:

- Date
- Plot code
- Hole No (example: #1)
- Depth
- Country

Further info:

If possible (and safe) the samples are air dried in the field (on camp), hotel room, local university, but this is not always possible. The samples taken from one expedition may not necessarily travel to the UK together. If one member of a team is returning to the UK before the end of the trip they may take the first samples with them or some samples may be shipped early before the end of the trip.

In the Labs:

It is possible that samples arriving at Geography Labs may be registered before all the samples taken on that expedition have arrived and thus may be registered with the labs and given a Job Number. This will be different to the Job number given to the rest of the samples when they arrive. I have tried to avoid this but it has happened.

Job Numbers (GL**) and School of Geography numbers (SOG****):**

The job numbers are not exclusively for tropical soils, every job that comes to the lab, whether it is for soil, peat, water, wood or leaves is given a job number, for whatever project (undergrad, postgrad, PhD, etc).

Registration:

When the samples arrive in the labs they are separated:

- Into plots (alphabetically)
- From each plot group they separated into pit or hole samples
- The pit samples are separated in to the Nutrient or Bulk Density samples
- The hole samples are separated into the separate holes (#1,#2 etc)
- They are then put into depth order.
- They are then allocated a SOG number, which is written on the bags and a Sample list is made, for example:

Job No	**SoG No**	Plot Code	Type(pit/hole)	Depth (cm)
GL3520	SOG049800	BKO-01	pit	0-5
GL3520	SOG049801	BKO-01	pit	5-10
GL3520	SOG049802	BKO-01	pit	10-20
GL3520	SOG049803	BKO-01	pit	20-30
GL3520	SOG049804	BKO-01	pit	30-50
GL3520	SOG049805	BKO-01	pit	50-100
GL3520	SOG049806	BKO-01	pit	100-150
GL3520	SOG049807	BKO-01	pit	150-200
GL3520	SOG049808	BKO-01	1	0-5
GL3520	SOG049809	BKO-01	1	5-10

GL3520	SOG049810	BKO-01	1	10-20
GL3520	SOG049811	BKO-01	1	20-30

8.2 University of Leeds Health and Safety protocols

STANDARD OPERATING PROCEDURES (SOPs)
FOR HANDLING AND DISPOSAL OF LICENSED MATERIAL
ACCORDING TO
PHL 104000/198559/3
Current licence
PHL 104000/198559/3 1st January 2016
Current Letter of Authority (LOA)
Issued 14th December 2017 - Validity 1st January 2018- 31st December 2018
Please Note
Current licence must be displayed on all soil containers and locations.
School of Geography Locations
Roger Stevens Level 5 Storage facility (room 5.52)
Garstang level 8: Soil lab (room 8.06)
Garstang level 8: Soil prep lab (room 8.06a)
Garstang level 8: R&D lab (room 8.08)
Garstang level 8: Cold room (room 8.02)
Garstang level 9: Analytical lab (room 9.08)
Garstang level 9: Water and Solvent lab (room 9.10)
Garstang level 9: Balance room (room 9.08a)
Garstang level 9: Cold room (room 9.02)
Garstang level 9: Controlled Temperature room (room 9.04)
Garstang level 9: Freezer (room 9.06)
APHA 001 List of Standard Operating Procedures Page Number

APHA 002 Introduction
The Faculty of Environment (Schools of Geography (SoG) and Earth and Environment (SEE)) is permitted by APHA to Import, Move and Keep Prohibited Soil for Chemical and Physical Analysis, from outside the EU provided the conditions outlined in the licence are adhered to. The current licence is **PHL 104000/198559/3**.
Prof Andy Dougill, Dr Tim Baker and Sarah Burdall are the nominated persons responsible for the licence. For the purposes of APHA records the SoG are classified as Project 1 and the SEE Project 2.
For the purpose of the licence EU Countries are:

- Austria
- Belgium
- Bulgaria

- Cyprus
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece
- Hungary
- Ireland
- Italy
- Latvia
- Lithuania
- Luxembourg
- Malta
- Netherlands
- Poland
- Portugal
- Romania
- Slovakia
- Slovenia
- Spain
- Sweden
- United Kingdom

For any enquiries regarding the SOP's and laboratory records for APHA licenced material please contact Rachel Gasior, Lab Manager, School of Geography, (r.l.gasior@leeds.ac.uk 33314) or Andy Connelly, Technical Officer, Cohen Laboratories, School of Earth and Environment, (a.connelly@leeds.ac.uk 30166).
For any enquiries regarding transfer of material to or from another institution in the UK contact the Faculty Health and Safety Manager (Sarah Burdall s.e.burdall@leeds.ac.uk 38042 / 30480).
For any enquiries regarding the plant health licence contact Mandy Riley via the Faculty Health and Safety Manager (Sarah Burdall s.e.burdall@leeds.ac.uk 38042 / 30480).
Animal and Plant Health Agency, Sand Hutton Site, York, YO41 1LZ, 01904 40150.

APHA 003 Transfer of Material to the UK
Samples should be packaged in such a way as to ensure they remain intact for the duration of the journey (triple containment. The use of sealed plastic containers fastened with cable ties is advised. To ensure sample traceability in the laboratory, each sample should be clearly labelled with the sample ID, country of origin and date of collection.
All consignments imported under the terms of this licence **must** be accompanied by a letter of authority as provided with this licence (to get a copy of this contact the Faculty Health and Safety Manager (Sarah Burdall s.e.burdall@leeds.ac.uk 348042). This letter must be contained within the package and displayed on the outside to prevent the package being opened at customs.
All consignments imported under the terms of the licence or obtained from other licensed sources in England and Wales **must** be conveyed directly from the place of landing or licensed establishment to the containment facilities authorised in this licence.

- For the School of Geography large consignments of samples should be delivered to room the Storage Facility Level 5, Roger Stevens Building for 'booking in'. Smaller consignments can be brought to the Soil Laboratory level 8 Garstang Building.
- For the School of Earth and Environment large consignments of samples should be delivered to Lab 9.133 Cohen Suite, Earth and Environment Building.

On arrival of the material all accompanying packaging material, any discarded materials and any extraneous soil must be disposed of in accordance with APHA/006.

Sample receipt

Receipt of material must be recorded in Sample Receipt Logbook;

- For the School of Geography this is located in the Technicians Office Garstang level 8.
- For the School of Earth and Environment this is located in the Technicians Office level 9, Cohen Suite .

The following information is to be recorded:

1. Date received
2. Principal investigator
3. Country of origin
4. Number of samples received

All import documentation must be forwarded to the Laboratory Manager

For the School of Geography

Rachel Gasior

Technicians Office, Level 9, Garstang Building

Tel 0113 343 3314

E-mail r.l.gasior@leeds.ac.uk

For the School of Earth and Environment

Andy Connelly

Technicians Office, Level 9, Cohen Suite, Earth and Environment Building

Tel 0113 343 30166

E-mail a.connelly@leeds.ac.uk

Any spillages must be dealt with in accordance with APHA 006 (Geography) or APHA 019 (SEE).

APHA/004 Storage of material

Long term storage

Soil samples for long term storage are held in;

- For School of Geography Storage Facility Level 5, Roger Stevens Building.

The room is equipped with an extraction system fitted with a HEPA filter, maintained and serviced annually by Estate Services. The external door entry to the building is fitted with a keypad and is accessible to all School of Geography members of staff; the internal door is lockable by key which is accessible to authorised personnel only.

- For School of Earth and Environment Cold room level 9 or in the locked cabinet room 9.138 SEE.

The cold room is under restricted access control to those with access to the level 9 labs and to the locked cold room. The cabinet in 9.138 is within a restricted access laboratory and kept locked with limited key access to authorised personnel.

Short term storage

Short term storage for samples being processed is:

- For School of Geography in the Soil Laboratory and the Soil Prep Laboratory Level 8 Garstang Building.
- For School of Earth and Environment in 9.138 Earth and Environment Building.

Storage Conditions
Licensed material is securely stored in triple containment, in self-seal bags stored within security containers. Each storage container is labelled to include the following information:
1. Soils Held Under Plant Health Licence
2. Date samples received
3. Job Number
4. Country of origin
5. Collection date
6. Sample IDs (range of IDs stored in the box)
7. Comments

APHA 005 Transfer of Material to Other Organisations
The material covered by this licence may be sent to persons or organisations who hold a current Plant Health Division licence **providing written agreement has first been obtained from Plant Health Division**. Material may also be sent to organisations overseas who have authority from their national plant health authorities to receive such material.
To transfer material to another organisation in the UK, contact the Faculty Health and Safety Manager (Sarah Burdall s.e.burdall@leeds.ac.uk 348042).

APHA 006 Decontamination of work surface and equipment and disposal of Material
Decontamination
All work areas and surrounding areas must be cleaned using a vacuum cleaner fitted with a HEPA filter and any remaining residue disinfected with 1% v/v Trigene.
Floors must be swept and disinfected with 1% v/v Trigene as necessary. All areas should be vacuumed and mopped with a solution of 1% v/v Trigene to remove contaminated dust.

Waste disposal (i.e. contaminated bags, gloves, containers etc)
All waste should be transferred to the **yellow** healthcare waste bags for incineration and transferred to the yellow lidded healthcare waste skip located in the secure healthcare waste store under the Staff Centre. Bags disposed of should be recorded in the log book in the store.

Records
Update the Sample Receipt Logbook to show the samples have been destroyed.

Contact
GW Butler via H&S services

Equipment
DeWalt DC500 Vacuum cleaner (or equivalent) fitted with a HEPA filter – there to be a Vacuum Cleaner in the laboratories and the Storage Facility Level 5, Roger Stevens Building.
APHA 007 Personal Protective Equipment (PPE) and Personal Hygiene
Dust masks
FFP2 Dust masks must be worn whenever there is a possibility of dust being generated as identified by the risk assessment or via a COSHH risk assessment e.g. soil sample weighing. Soil sample preparation (sieving and grinding must be carried out in the microbiological safety cabinet). Where the use of a face mask is identified the individual will need face fit

testing (Contact Health and Safety Manager for information Sarah Burdall s.e.burdall@leeds.ac.uk 38042).

Vinyl / Nitrile gloves
Vinyl or Nitrile gloves must be worn at all times when handling soil from outside the EU.
Goggles
Goggles must be worn as identified in the COSHH risk assessment.

Laboratory Coats
A laboratory coat must be worn at all times when handling soils from outside the EU. Coats must be stored in laboratory.

Ear Defenders
Ear defenders must be worn as identified by the noise risk assessment when using the MM301 mixer mill, the risk assessment will identify the appropriate SNR level for the ear defenders. Where exposure to noise is identified then a base line hearing test will need to be carried out (Contact Health and Safety Manager for information Sarah Burdall s.e.burdall@leeds.ac.uk 38042).

Disposal of used PPE
Consumable items must be disposed of in accordance with APHA/006.

Personal Hygiene
Wash hands with soap and hot water before leaving the laboratory.
Food Drink and Personal items are not to be taken into the laboratory.

APHA 008 Authorised Personnel
Please refer to Appendix 1 for the current list.

APHA 009 Authorised Locations

APHA 010 Cleaning Vacuum HEPA Filter
When the efficiency of the vacuum is no longer adequate replace the HEPA filter. Discard the contents of the tank and the used filter as per APHA/006.

For the School of Geography and School of Earth and Environment
Procedure
1. Press the On/Off power button on the biological safety cabinet once to start the blower
2. Place the vacuum in the biological safety cabinet
3. Remove the filter and transfer to an autoclave bag.
4. Empty the contents of the vacuum tank to an autoclave bag.
5. Replace the vacuum filter
6. Disinfect the safety cabinet following as per APHA/006.

Replacement filters can be purchased from tlc-direct.co.uk part number DW DC500 1XJ.

APHA 011 Use of Microbiological Safety Cabinet
For the School of Geography
The microbiological safety cabinet should be used when samples have been exposed to agricultural, animal or human waste, or rotting material of animal origin and for activities

where dust is generated. Bookings can be made using the Microsoft Outlook Calendar: Public folders/Geography/Lab Instrument bookings/biological safety cabinet.

Operation
1. Press the On/Off power button once to start the blower,
2. If the UV light is required press the On/Off button,

NB the UV light can only be operated when the sash is closed,
3. After use turn off the blowers and lights,

Daily User Maintenance
4. Wipe the stainless steel surfaces with a solution of 1% v/v Trigene, Do not use chlorinated disinfectants or solvents,
5. Check the airflow before use, the ideal flow rate should ~ 0.5 m/s,
6. Vacuum all areas in the cabinet after use paying particular attention to the pre-filter at the back of the cabinet.

Engineer Maintenance
The cabinet is inspected and tested annually by **Howarth Airtech or Triple Red.**

APHA 012 Use of autoclave
For the School of Geography
Model LTE Touchclave-R 160H bench top autoclave or equivalent, this autoclave has been validated for the loads using a thermocouple test. The autoclave must only be used by trained operatives.

Laboratory Procedure
1. If available attach a strip of indicator tape to the autoclave bag (indicator strip must be capable of changing colour after 30 minutes at 121 □C). Alternatively, reset the pressure max indicator to zero (the red needle on the pressure display).
2. Tie a strip of autoclave bag around the top of the bag to act as a closure.
3. Follow the procedure below for autoclave operation:

Autoclave operation
i. Check that 'POWER' lamp is on.
ii. Close door and lock.
iii. If display shows "tank water level low", add tap water to the tank.
iv. Press 'OPEN' button. After 30 seconds the bolt will unlock.
v. Turn handle clockwise and open the door.
vi. Load autoclave.
vii. Close door and rotate handle anticlockwise to lock.
viii. Display changes to normal display showing ready to start "LOCKED" lamp will be on.
ix. Select the appropriate program: 'DESTRUCT' for dry material and 'LIQUIDS' for liquid samples.
x. Press 'START' button. Program will begin.
xi. When display shows "COMPLETE" at the end of the cycle, press 'OPEN' key.
xii. After 30 seconds, when door unlocks rotate the handle and open the door.

4. When the cycle is complete remove the autoclave bag from the chamber check that the indicator tape has changed colour or that the pressure max indicator has reached the required pressure.

5. If the autoclave has worked successfully the indicator strip should have changed colour and/or the pressure max indicator shows a value of > 1 bar.
6. Transfer waste to the yellow healthcare waste disposal bags for incineration.
7. If the autoclave has failed to reach the required settings contact Laboratory Servicing for advice. In the event of failure store the waste in a sealed container until the unit can be repaired.

Daily User Maintenance
1. Carry out visual inspection and testing of door interlocks
a. Unlock and open the door
b. Check for smooth swinging and/or lifting action with no grating or crunching noises, or looseness
c. Inspect the locking pins. They should slide freely as the handle is moved
d. Operate the door handle. Observe the operation of the bolts. Operation should be firmly limited by the end stops, and all bolts must operate in the same manner. Check that the solenoid locking pin operates when handle is in fully locked position (press 'OPEN' to release lock)
e. Close door and hold shut do not lock display should change to 'UNLOCKED'
f. Rotate handle, lock and check that locking pin functions correctly and prevents unlocking. Display should show the normal display as if ready to start
g. Use 'OPEN' button to unlock

Monthly User Maintenance
1. Clean the chamber taking care to avoid sensors
2. Release the Hoffman clip on the drain pipe and allow the tank to drain, refill the tank with tap water
3. If necessary clean the tank level sensor using fine sand paper

Engineer Maintenance and Statutory Pressure System Inspections
A statutory pressure system inspection is carried out annually by Allianz Engineering; records of this are kept with:

- School of Geography Mr Robert Finch
- School of Earth and Environment Mr Jerry Lee

Autoclaves are serviced annually