

SOIL ANALYSIS METHODS AND RESULTS INTERPRETATION MANUAL

A GUIDE FOR SOIL LABORATORIES IN PACIFIC ISLAND COUNTRIES AND TERRITORIES

Land Resources Division

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Introduction

Most Pacific Island soil laboratories are still using the manual developed by Daly and Wainiqolo (1993a) to carry out and interpret results of soil analyses. However, these laboratories have participated in soil proficiency tests organised by the Australasian Soil and Plant Analysis Council (ASPAC) for the Pacific region; and the laboratories have been accredited to ASPAC, which is an active member of the Global Soil Laboratory Network (GLOSOLAN). ASPAC is validating proficiency results of soil analyses using methods developed by Rayment and Lyons (2011). Although methods provided in the two soil analysis manuals are similar, those of Rayment and Lyons (2011) are more up to date than those of Daly and Wainiqolo (1993a).

Although Pacific Island soil laboratories have access to the methods of Rayment and Lyons (2011), these have not been adopted because they are not written as simply as those of Daly and Wainiqolo (1993a). ASPAC has encouraged the use of Rayment and Lyons (2011) methods for regional soil analysis and results interpretation so that results are comparable to those of Australia and New Zealand. Therefore, there is an urgent need to simplify the methods of Rayment and Lyons (2011) by writing them in a format that Pacific Island soil laboratory technicians can easily understand. This was evident when consultations were held with the Pacific Island soil laboratories and technicians, and researchers requested to rewrite Rayment and Lyons (2011) methods step-by-step, similar to those of Daly and Wainiqolo (1993a).

This manual serves as an initial step in simplifying the analysis methods of Rayment and Lyons (2011). It focuses on basic soil chemical properties – pH, electrical conductivity, soil organic carbon, and soil nutrients, namely nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and boron (B). Methods to determine these properties, and interpretation of results, are provided in this manual. This set of guidelines also includes other soil analytical protocols to produce a complete soil analytical manual. The soil analysis (testing) methods described in this laboratory guide are the more commonly used methods applicable to most soil types. To assist manual users, interpretation data for analysis results are given for each method. However, it should be noted that the methods of soil nutrient analysis described are yet to be validated under Pacific Island laboratory conditions.

1.0 Soil sample preparation (Method 102)

1.1 INTRODUCTION

Soil samples collected for chemical analysis must be dried before conducting the analysis. This is for ease of handling, to allow comparison between samples of different water contents, and to enable the sample to be ground. Most results are quoted on an oven-dry (105°C) basis, however analyses are carried out on air-dry samples (dried at a temperature not higher than 40°C), as oven drying causes changes in several chemical properties. Oven drying applies a moisture factor to results calculation (see next section).

Samples are ground so that representative subsamples can be taken even when small sample weights are used for analyses. Coarse grinding to < 2 mm is sufficient for most analyses. Fine grinding to < 0.25 mm is used for analyses where less than 1 g sample is used (total carbon and nitrogen). Fine grinding is not necessary if these analyses have not been requested.

1.2 APPARATUS

- 1.2.1 FORCED-AIR DRYING CABINET, with controllable temperature.
- 1.2.2 ROLLER MILL, with 2 mm sieve grinding drum, for coarse grinding.
- 1.2.3 RING GRINDER OR MORTAR AND PESTLE, for fine grinding.

1.3 PROCEDURE

1.3.1 REGISTRATION

- 1.3.1.1 Sort samples into the order of field or client number.
- 1.3.1.2 Assign a lab number to each sample and record job number, lab number, client number and analyses requested in the register.

1.3.2 DRYING

- 1.3.2.1 Empty the soil sample into a drying tray and add a paper label with the lab number.
- 1.3.2.2 Break up the soil into less than 1 cm lumps and mix. Remove any insects and as much organic matter as possible that was live at the time of sampling (e.g. live roots).
- 1.3.2.3 If necessary, take subsamples for particle size determination. If the sample is very large a portion can be discarded after mixing.

1.3.2.4 Place the tray in the dryer, set at not more than 40°C and leave until dry. This usually takes 48 hours.

1.3.3 GRINDING AND SUBSAMPLING

1.3.3.1. Label two sample containers (500 mL container for < 2 mm sample and 100 mL container for < 0.25 mm sample) with the lab number.

1.3.3.2 Take the soil out of the dryer and pour into the drum of a grinder. The drum should have large and small stainless-steel rollers. For soils with high clay content, it may be necessary to use three rollers. Switch on the grinder.

1.3.3.3. All of the soil, including concretions, must be ground, but not stones. If stones are present, they will need to be separated from the soil before it is crushed. Sieve out the soils and put the soil collected back into the grinder.

1.3.3.4 Thoroughly mix the ground soil.

1.3.3.5 Carefully subsample and fill the large and the small sample containers. Discard excess.

1.3.4 FINE GRINDING

1.3.4.1 Pour soil from the small sample container into the head of the ring grinder.

1.3.4.2 Grind for 10 seconds to approximately < 0.25 mm. It is preferable but not necessary to use a sieve.

1.3.4.3 Brush out the sample and return it to the small sample container.

1.3.4.4 Between samples, clean the grinder using a paper towel to wipe the inside of the head and rings to ensure that there is no carry-over material. Where the soil is sticky, the head and rings will need washing between samples. Wash using a plastic brush, rinse with distilled water and dry before the next sample.

1.3.5 CLEANING UP

1.3.5.1 When all samples in the set have been ground, wash all the grinding heads, rollers, etc. in tap water, rinse with distilled water and dry well before storing.

1.3.5.2 Wash dryer trays and dry in drying cabinets.

1.3.5.3 Wipe out roller mills and wipe down benches with a damp cloth.

2.0 Moisture factor (Method 104)

2.1 INTRODUCTION

Most results of soil chemical analyses are quoted on an oven-dry (105°C) basis, but as oven drying causes changes in several chemical properties, analyses are carried out on air-dried samples (dried at a temperature of not more than 40°C). To convert results to an oven-dry basis, a moisture factor is applied in the calculation of results.

2.2 PROCEDURE

- 2.2.1 All weightings should be in grams to three decimal places (0.001 g accuracy).
- 2.2.2 Weigh a labelled aluminium dish, approximately 7 cm in diameter, with a lid, and record the weight (W_1).
- 2.2.3 Weigh accurately a 10–20 g sample of soil (air-dry, <2 mm) in the dish with lid and record the weight (W_2).
- 2.2.4 Dry the dish with lid in an oven at 105°C for 8–24 hours.
- 2.2.5 Remove the dish from the oven, fit lid, cool and weigh (W_3).

Note: Because oven-dry soil rapidly picks up water vapour from the atmosphere (even in some desiccators), it is necessary to carry out all weighing as soon as the dish is cool enough to handle, but before it cools to room temperature. This can be achieved, if about six dishes are removed from the oven at a time, placed on a tray and taken directly to the balance and weighed.

2.3 CALCULATION OF RESULTS

$$\begin{aligned}\text{Moisture factor} &= \frac{\text{weight of air-dry soil}}{\text{weight of oven-dry soil}} \\ &= \frac{(W_2 - W_1)}{(W_3 - W_1)}\end{aligned}$$

Report results to 3 decimal places.

3.0 pH of 1:5 soil:water suspension (Method 4A1)

3.1 INTRODUCTION

Soil pH is one of the most important and easiest soil chemical characteristics to determine. Soil pH alone has little direct effect on crop production. However, it correlates with many other soil properties such as base saturation. Therefore, it has a very strong direct effect on several other soil properties that affect crop production. Soil pH controls the availability of many plant nutrients, with low or very high pH causing low availability for nutrients such as phosphorus and many trace elements. At pH below 5.6, toxic levels of exchangeable aluminium can be present in the soil.

The soil acidity is measured in moles of H^+ ions L^{-1} of solution. The pH scale is used to express acidity, where the pH value is the negative logarithm of the moles of H^+ ions L^{-1} of solution. The pH scale ranges from 0 to 14, with 7 neutral, below 7 acidic, and above 7 alkaline. For most soils, the pH range is 3–9. The desirable soil pH range for optimum plant growth varies among crops. Generally, a soil pH between 6.0–7.5 is acceptable for most plants, as most nutrients are available in this pH range (FAO 2021).

Soil pH may be measured electrometrically with a pH meter. Water is the usual suspension medium for the soil sample, but 1 M ($mol.L^{-1}$) KCl or 0.01 M $CaCl_2$ are also used, particularly to minimise the effect of soluble salt. This gives lower values for most soils as the potassium or calcium ions replace acidic groups (positively charged H and Al ions) from cation exchange sites on soil clay particles. For some soils, 1M KCl pH is higher than water pH as the soil clays have a net positive charge and chloride ions from the salt replace hydroxyl groups from the ion exchange sites. KCl pH is measured to identify such soils.

Ratios of soil to water used to measure pH, range from 1:1 to 1:10 with 1:2.5 being the most common. The ratio used here is 1:5 as it allows electrical conductivity (EC) to be measured on the same extract and for the large majority of soils 1:5 gives the same result as 1:2.5.

3.2 REAGENTS

3.2.1 DISTILLED OR DEIONISED WATER. This water is to be in equilibrium with atmosphere with respect to CO_2 concentration, and should have an EC of $<1.5 \times 10^{-3}$ dS/m.

3.2.2 BOILED DISTILLED OR DEIONISED WATER FOR THE BUFFER SOLUTIONS. This water should have a pH ≥ 6.5 but ≤ 7.5 . This can be obtained by boiling distilled or deionised water for 15 minutes and cooling under CO_2 -free conditions. Its EC should be $<10^{-3}$ dS/m (Alvarez 1984).

3.2.3 pH 4.00 BUFFER (at 25°C). Use a purchased solution. Alternatively, prepare a 0.0496 M solution by dissolving 10.12 g potassium hydrogen phthalate ($KHC_8H_4O_4$; previously dried for 2 h at 110°C) and fill the volume to 1.0 L with deionised water described for use with buffer solutions (Alvarez, 1984). Specific conditions to exclude CO_2 are unnecessary but protect against evaporation and contamination. Store for up to one month but discard solution if mould appears.

- 3.2.4 pH 6.86 BUFFER (25°C). Use a purchased solution. Alternatively, dissolve 3.387 g potassium dihydrogen phosphate (KH_2PO_4) and 3.533 g disodium phosphate (Na_2HPO_4) in deionised water and make to 1.0 L with deionised water described for use with buffer solutions. Dry the chemicals for 2 h at 110–130°C before use. Store for up to one month in chemical-resistant glass and protect from CO_2 , evaporation and contamination, but discard solution if mould appears.
- 3.2.5 pH 7.0 BUFFER (25°C). Use a purchased solution. Alternatively, dissolve 2.721 g potassium hydrogen phosphate (K_2HPO_4 ; previously dried for 2 h at 130°C) and 3.904 g anhydrous disodium phosphate (Na_2HPO_4 ; previously dried at 130°C for 2 h) and make to 1 L with deionised water, as described for use with buffer solutions. These correspond to 0.020 M KH_2PO_4 and 0.0275 M Na_2HPO_4 . Protect solution from CO_2 , evaporation, and contamination. Store for up to one month but discard solution if mould appears.
- 3.2.6 pH 9.183 BUFFER (25°C). Use a purchased solution. Alternatively, dissolve 3.80 g sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, stored in a desiccator over a saturated aqueous solution of NaCl and sucrose) and make to 1.0 L with deionised water described for use with buffer solutions. (Note that $\text{Na}_2\text{B}_4\text{O}_7$ may lose water of crystallisation during long storage in a chemical store). Add a small crystal of thymol ($\text{C}_{10}\text{H}_{14}\text{O}$) to prevent growth of microorganisms. Storage of the bulk buffer in a sealed container for up to one month should be possible. Prevent absorption of atmospheric CO_2 and use within 10 min of pouring into an open container.

3.3 PROCEDURE

- 3.3.1 Prepare a 1:5 soil:water suspension. For example, weigh 20.0 g air-dry soil (< 2 mm) into a suitable bottle or jar and add 100 mL deionised water. Close the bottle.
- 3.3.2 Include a reference sample through the procedure.
- 3.3 Mechanically shake, end-over-end, at 25°C for 1 h.
- 3.3.4 Allow at least 20–30 min, but preferably 4 h, for the soil to settle and make all measurements. All the measurements must be made on the same day of extraction.
- When soil EC (method 3A1), water soluble Cl^- , and/or water soluble NO_3^- are also required, EC should be measured first and aliquots taken for pH, water soluble Cl^- and NO_3^- .
- 3.3.5 Standardise the pH meter according to the manufacturer's instructions, using the buffer at pH 6.86 or pH 7.0, and either the pH 4.0 or pH 9.183 buffer depending on the expected values of the soils. Stir these buffer solutions with a mechanical stirrer during measurement.
- 3.3.6 Thoroughly wash the electrodes between measurement of buffer solutions and soil solutions/extracts.
- 3.3.7 When measuring soil suspensions pH, ensure electrodes are well immersed.
- 3.3.8 Record the pH value obtained when the meter appears steady while the suspension is being mechanically stirred. Replicate determinations should give results within 0.1 pH unit.

3.3.9 Report pH (1:5 soil:water) on an air-dry basis.

Notes:

1. The use of three buffers during calibration provides a check on the linearity of electrode response.
2. When soil pH values >10 are expected, use a glass electrode designed for highly alkaline conditions.
3. Occasionally confirm whether there is adequate leakage of KCl from the calomel electrode, otherwise readings may be inaccurate. This is done by placing the calomel electrode in 10 mL deionised water for 1 min before testing for the presence of Cl^- with AgNO_3 .

Interpretation of results

Table 1. pH rating and interpretation (from Daly and Wainiqolo 1993b)

Rating	pH	Description
Very high	>9.0	Extremely alkaline
	8.5–9.0	Strongly alkaline
	7.9–8.4	Moderately alkaline
High	7.3–7.8	Slightly alkaline
	6.7–7.2	Near neutral
Medium	6.1–6.6	Slightly acidic
	5.6–6.0	Moderately acidic
Low	4.4–5.5	Strongly acidic
Very low	<4.4	Extremely acidic

4.0 Electrical conductivity (Method 3A1)

4.1 INTRODUCTION

Soluble salts content in soil is determined by measuring the electrical conductivity (EC) of the soil solution. The EC provides a measure of the soil extract's capacity to transmit an electric current. Electrical conductivity is generally related to the total solute concentration and can be used as a quantitative expression of dissolved salt concentration. The main causes of accumulated soluble salts in soils are heavy and improper fertiliser use, irrigating with salt-laden water, sea water intrusion or pedogenic processes (Rayment and Lyons 2011).

The term salinity refers to the presence of the major dissolved inorganic solutes, essentially Na^+ , Mg^{2+} , Ca^{2+} , K^+ , Cl^- , SO_4^{2-} , HCO_3^- and CO_3^{2-} in aqueous samples, which is measured through electrical conductivity. EC determination is often sufficient for diagnosing, surveying and monitoring soil salinity, and for assessing the adequacy of leaching and drainage (FAO 2021).

EC is measured with varying soil:water ratios. Common ratios include 1:1, 1:2.5 and 1:5 in addition to saturation extracts. The ratio 1:5 has wide acceptance and is described here. This ratio is the same as that used for pH, and the EC should be measured before pH measurement on the same extract. The suspension is not filtered before measurement as suspended particles have a negligible effect on the conductivity, and it is more important to make the measurement quickly, as microbiological activity can change the ionic concentration.

4.2 REAGENTS

- 4.2.1 DEIONISED WATER. This water should have an EC of $<10^{-4}$ dS/m, and a CO_2 concentration in equilibrium with the atmosphere.
- 4.2.2 ACID DICHROMATE CLEANING SOLUTION. To 32 mL of saturated water solution of sodium dichromate ($\text{Na}_2\text{Cr}_2\text{O}_7$) add 1.0 L sulphuric acid (H_2SO_4 ; 18 M). *Handle with caution as this solution is both corrosive and a strong oxidant.*
- 4.2.3 0.1 M POTASSIUM CHLORIDE REFERENCE SOLUTION. Dissolve 0.7455 g potassium chloride (KCl; previously dried at 110°C for 2 h) and make volume to 1.0 L with deionised water that is free of CO_2 . This solution has an EC of 1.413 dS/m at 25°C .

4.3 PROCEDURE

3.1 EXTRACTION

- 3.1.1 Prepare a 1:5 w/v soil:water suspension. For example, weight 20 g of air-dry soil into a suitable bottle or jar and add 100 mL deionised water. Close the bottle.
- 3.1.2 Include a reference sample through the procedure.

3.1.3 Mechanically shake (end-over-end preferred), at 25°C for 1 h to dissolve soluble salts.

3.1.4 Allow about 20–30 min for the soil to settle.

3.2 MEASUREMENT

3.2.1 Calibrate the conductivity cell and meter according to the manufacturer's instructions, using the KCl reference solution at the temperature of the suspensions.

3.2.2 Dip the conductivity cell into the settled supernatant, moving it up and down slightly without disturbing the settled soil.

3.2.3 Take the reading with the cell stationary when the system has stabilised. Rinse the EC cell with deionised water between samples and remove excess water.

Complete EC measurements within 3–4 h of obtaining the aqueous supernatant. Reference soils should be included in each batch of unknown samples.

3.2.4 Report EC (dS/m) at 25°C on an air-dry (40°C) basis.

Notes:

1. High quality RODI (reverse system deionization) that contains inconsequential traces of soluble organic matter is preferred for reagents and Standard Solutions. This equates to the American Society for Testing Materials (ASTM) Type 1 grade of reagent water.
2. If EC readings become erratic, clean the EC electrode by soaking it in acid dichromate cleaning solution overnight, followed by thorough rinsing with deionised water. If the platinum black has flaked, recoat according to the procedure outlined in APHA (1998). Rinse electrodes thoroughly and keep immersed in water when not in use.
3. The depth of insertion of the EC electrode should be checked against the 0.01 M KCl Reference Solution to determine locations where no effect on the correct reading occurs. With unshielded electrodes, small containers may be unsuitable for use.

Interpretation of results

Table 2. EC rating and interpretation (from Daly and Wainiqolo 1993b)

Rating	EC _{1:5} (dS/m)
Very low	<0.15
Low	0.15–0.4
Medium	0.4–0.8
High	0.8–2.0
Very high	>2.0

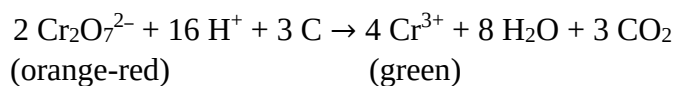
5.0 Organic carbon (Method 6A1)

5.1 INTRODUCTION

Soil carbon (C) is the smallest but the most important component in soils, as it affects almost all soil physical, chemical and biological properties. Soil organic matter is a primary indicator of soil quality. Improvements in soil organic matter create a more favourable soil environment, leading to increases in plant growth. Higher soil organic matter levels allow the soil to retain more water, which results in better crop yields, reduces soil erosion, increases plant nutrient retention, increases biological diversity. Moreover, improved aggregation of soil particles results in better soil structure, allowing for movement of air and water through the soil, as well as better root growth. More stable soil structure results in less soil erosion, which retains nutrients on the land and protects water quality. Soil organic C contributes to the cation exchange capacity of a soil. These cation exchange sites are important for retention of nutrients such as calcium, magnesium and potassium. Soil organic C often also provides binding sites for many anthropogenic organo chemicals, thus minimising leaching of hazardous chemicals through the soil profile or making them less bioavailable, which reduces their toxicity. Increased soil organic C enhances the biomass and diversity of the soil biota (FAO 2019).

Organic C in soil is determined with dry or wet oxidation methods. Dry oxidation with automated CN analysers has the advantage that all C forms in the soil are included in the measurement and that nitrogen and eventually other elements can be measured simultaneously. Procedures for wet oxidation in acid dichromate solution are available with or without external heat. In the latter case, the oxidation of organic matter is incomplete, and a correction factor is applied, such as assuming that on average only 77% of organic C has been oxidised. As C recovery varies between soils in a usually unknown manner, this method should only be used for treatment comparisons with the same soil, and not across different soil types. Wet oxidation largely excludes elemental C, such as charcoal, which can be an advantage in some situations, such as when studying the effect of organic matter management in soils that contain large and/or variable amounts of charcoal. The importance of charcoal as a component of stable organic matter in soils is increasingly recognised.

The method described here is an adaptation of the Walkley and Black (1934) procedure, in which the soil organic matter is oxidised by dichromate ($\text{Cr}_2\text{O}_7^{2-}$) and sulphuric acid using the heat of dilution of the sulphuric acid (about 110–120°C), which is sufficient to induce substantial oxidation. The reaction is as follows:



In the absence of interference, the chromic ions (Cr^{3+}) produced should be reasonably proportional to the oxidised organic C (OC). In aqueous solution, Cr^{3+} has maximum absorption at 450 nm and 600 nm. Since $\text{Cr}_2\text{O}_7^{2-}$ does not absorb at 600 nm, the absorbance at this wavelength can be used to estimate Cr^{3+} and hence OC. An advantage over the original titrimetric procedure and the colorimetric estimation of unreacted $\text{Cr}_2\text{O}_7^{2-}$ solution is that accurate standardisation of the $\text{Cr}_2\text{O}_7^{2-}$ solution is not required.

5.2 REAGENTS

- 5.2.1 REAGENT WATER. This water should be in equilibrium with atmospheric CO₂ concentration, it should have an EC of $< 1.5 \times 10^{-3}$ dS/m, and it should be devoid of soluble organic matter sufficient to affect the blank.
- 5.2.2 0.5 M SODIUM DICHROMATE. Dissolve approximately 149 g L. R. sodium dichromate (Na₂Cr₂O₇·2H₂O) in reagent water and make volume to 1.0 L. Filter through sintered glass or glass-fibre filter material to ensure the solution is free of particles prior to use.
- 5.2.3 1.0 M CHROMIUM TRIOXIDE (alternative to 0.5 M sodium dichromate). Dissolve ≈100 g L.R. chromium trioxide (CrO₃) in Reagent Water and make volume to 1.0 L. Filter through sintered glass or glass-fibre filter material to ensure the solution is free of particles prior to use.
- 5.2.4 STANDARD SUCROSE SOLUTION. 1 mL contains 5 mg of C. Dissolve 11.8745 g sucrose [(C₁₂H₂₂O₁₁), previously dried for at least 24 h in a desiccator over sulfuric acid (H₂SO₄; 18 M)], and dilute to 1.0 L with Reagent Water in a volumetric flask.
- 5.2.5 SULPHURIC ACID. Commercial sulfuric acid (H₂SO₄; sp. 1.84), free of suspended matter.

5.3 PROCEDURE

Non-saline soils

- 5.3.1 Prepare a series of standards for each set of analyses by dispensing 0, 1.0, 2.0, 3.0, 4.0, 5.0, ... 10.0 mL (as required) of the Standard Sucrose Solution into 250 mL conical beakers. These standards contain 0, 5, to 50 mg C, corresponding to 0–50% C for a 1 g soil sample and 0–25% C for a 0.2 g sample.
- 5.3.2 Evaporate the dispensed sucrose solutions to dryness in an oven at not greater than 65°C then cool to room temperature.
- 5.3.3 Weigh samples of finely-ground (<0.5 mm), air-dry soil, according to the expected C content: 1.00 g for expected values of <5% C, and 0.2 g for expected values >5.0% C.
- 5.3.4 Include blank samples throughout the procedure.
- 5.3.5 To the standards and soils add 10 mL of 0.5 M sodium dichromate or 1.0 M chromium trioxide solution and swirl gently to ensure all soil particles are wetted.
- 5.3.6 Wait 10 min with occasional swirling, then carefully but quickly add across 10–15 sec (use gentle swirling to avoid the loss of soil and chromic acid from localized boiling) 20 mL concentrated H₂SO₄.
- 5.3.7 Wait a further 30 min with occasional swirling, then add 170 mL Reagent Water from a dispenser, stir or swirl to mix thoroughly and set aside to cool for particles to settle.

Consistent timing for each H₂SO₄ addition and diluent water helps ensure constant reaction conditions, including heat generation.

- 5.3.8 After cooling, centrifuge if the supernatant is not clear.
- 5.3.9 Determine absorbance of the supernatant at 600 nm with Reagent Water set at zero.
- 5.3.10 Construct a standard curve by plotting absorbance of the standard sucrose assays against their known contents of C (or use a microprocessor-controlled equivalent).
- 5.3.11 Dispose of spent reagents and treated soils in a safe and environmentally responsive manner, noting that Cr is a heavy metal that is both toxic and environmentally persistent.
- 5.3.12 Repeat the determination with less soil if >75% of $\text{Cr}_2\text{O}_7^{2-}$ is reduced (only likely with soils of very high C content).

Saline soils (>0.5% Cl)

- 5.3.13 Determine the water-soluble Cl (Method 5A1 or 5A2) and express results for Cl as a percentage, noting that 10 000 mg Cl/kg = 1% Cl. Also determine apparent $\text{OC}_{\text{W\&B}}$ as described for non-saline soils. Again, repeat the determination with less soil if >75% of $\text{Cr}_2\text{O}_7^{2-}$ is reduced.

CALCULATION (for saline soils only)

$$\text{OC}_{\text{W\&B}} = (\text{Apparent } \text{OC}_{\text{W\&B}} (\% \text{ C}) - \frac{1}{12} \text{water-soluble Cl}^{-} (\% \text{ Cl}))$$

both expressed on the same soil moisture status and allowing for the weight of the sample.

Report $\text{OC}_{\text{W\&B}} (\% \text{ C})$ on an oven-dry basis. Use the air-dry moisture to oven-dry moisture ratio to convert an oven-dry concentration.

Interpretation of results

The accuracy of obtained data in the laboratory is a precondition for a reliable interpretation. The following are some broad ratings of soil organic C (Blakemore et al. 1987).

Table 3. Ratings for Organic C

Rating	Organic C (%)
Very high	>20
High	10–20
Medium	4–10
Low	2–4

Very low	<2
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6.0 Nitrogen (Method 7A1)

6.1 INTRODUCTION

Nitrogen (N) occupies a prominent role among nutrient elements because of the relatively large amounts that are required by plant and soil micro-organisms in comparison to other elements. Low N availability limits crop growth in many tropical soils. More than 95% of N in the topsoil is usually present in organic forms. Through microbial mineralisation, it is transformed into ammonium (NH_4^+) and then further oxidised by nitrifying soil microbes to nitrite (NO_2^-) and nitrate (NO_3^-), a process called nitrification. Plants absorb most of their N from the soil as NH_4^+ or NO_3^- , with nitrite being present only in minor quantities in well-aerated soils such as most Pacific Island soils. However, both trees and herbaceous plant species have the ability to take up certain organic N forms, especially when they are associated with mycorrhizal fungi. The burning of vegetation, as in the shifting cultivation of the South Pacific, can lead to large losses of biomass N. As NO_3^- , N is also easily lost from agricultural soils through leaching, especially following the mineralisation peak. Nitrogen losses can also occur through denitrification and volatilisation of NH_3 , for example from decomposing biomass and manure or surface-applied fertiliser, especially urea.

The methods for the measurement of total N in soil and plant materials are based either on wet oxidation or dry combustion. The wet oxidation method is based on the Kjeldahl digestion procedure (Kjeldahl, 1883) as modified by Bremner and Mulvaney (1982) with sulphuric acid and a catalyst. This is popularly known as the semi-micro Kjeldahl method and is the most widely used method to determine total N throughout the developing world, including the Pacific Islands. A problem with the method is that some but not all, NO_3^- in the soil is included in the measurement. The dry combustion method is used in automated CN analysers. Their advantage is the inclusion of all mineral and organic N forms.

In the semi-micro Kjeldahl method, organic and chemically combined N is oxidised by a catalyst, with heating to $\approx 420^\circ\text{C}$, and subsequently altered to NH_4^+ , usually by the micro-Kjeldahl process. Further, N is converted to NH_4^+ -N by digestion with concentrated sulphuric acid, sodium sulphate to raise the boiling point, and copper as a catalyst to convert nitrogen in the sample to ammonium sulphate. The NH_4^+ -N is determined in the digest by releasing the ammonia from the alkaline solution by steam distillation, collection in dilute boric acid followed by titration with hydrochloric acid. This method extracts some of the interstitial NH_4^+ held in clay lattices, but in most tropical soils the error thus introduced is probably very small.

6.2 APPARATUS

6.2.1 DIGESTION TUBES, 50-mL calibrated glass tubes.

6.2.2 ALUMINIUM HEATING BLOCK, $220 \times 220 \times 50$ mm drilled with four rows of five holes (27 mm diameter, 35 mm depth).

- 6.2.3 HOT PLATE, domestic stove style single radiant element with a diameter of about 200 mm and an output of about 2 kW, mounted on a steel frame and fitted with a simmerstat.
- 6.2.4 STEAM DISTILLATION ASSEMBLY, glass system such as Parnas-Wagner or Markham, or semi-automated system such as Bucchi.
- 6.2.5 BURETTE, readable to 0.05 mL.

6.3 REAGENTS

- 6.3.1 DIGESTING ACID. Commercial grade sulfuric acid (H_2SO_4 ; 18 M).
- 6.3.2 KJELDAHL COPPER CATALYST TABLETS, BDH Cat No. 33064. Each tablet contains 1 g sodium sulphate and 0.1 g copper sulphate. Alternatively prepare a mixture of 1.0 kg anhydrous sodium sulphate (Na_2SO_4) and 100 g of anhydrous copper sulphate (CuSO_4) after first grinding each component separately.
- 6.3.3 60% SODIUM HYDROXIDE SOLUTION. Dissolve 600 g commercial or technical grade sodium hydroxide (NaOH) in deionised water, cool and make to 1.0 L.
- 6.3.4 2% BORIC ACID. Dissolve 20.0 g H_3BO_3 in about 800 mL of water, heating if necessary. Add water to make 1 L of solution. Adjust pH to 5.0 with diluted NaOH immediately prior to use. If ≤ 5000 μg of N needs to be fixed, 1% H_3BO_3 aqueous solution (10.0 g $\text{H}_3\text{BO}_3/\text{L}$) can be substituted to increase the sharpness of the end point.
- 6.3.5 BROMOCRESOL GREEN–METHYL RED MIXED INDICATOR. Mix 100 mL 0.1% bromocresol green [0.1 g bromocresol green ($\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$) dissolved in 100 mL 95% ethanol ($\text{C}_2\text{H}_5\text{OH}$)] with 20 mL 0.1% methyl red [0.1 g methyl red ($\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_2$) dissolved in 100 mL 95% ethanol]. Proportions may need to vary slightly to obtain the neutral-grey transition colour of the indicator. *Those who suffer from colour blindness may need to use an alternative indicator with more obvious colour change characteristics.*
- 6.3.6 0.010 M HYDROCHLORIC ACID

1 mL = 0.1401 mg of N.

To prepare 10 L, dilute 10.0 mL hydrochloric acid (HCl ; 10 M) with deionised water, cool and make up to volume. Standardise against sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), which has been stored in a desiccator over an aqueous saturated solution of NaCl and sucrose for at least 24 h. Weigh out 0.9535 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, dissolve in deionised, CO_2 -free water and make up to 500 mL. Titrate 25 mL aliquots with the 0.01 M HCl using the mixed indicator (Alvarez 1984).

$$\text{Molarity of HCl} = \left[\frac{\text{g of Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O in 500 mL}}{190.69} \times \frac{\text{aliquot}}{500} \times \frac{1000}{\text{titre}} \right]$$

Note that 0.005 M H_2SO_4 may substitute for 0.010 M HCl and is preferred for ^{15}N analyses. Also, alternatives to $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ can be substituted for standardisation purposes.

6.4 PROCEDURE

6.4.1 DIGESTION

- 6.4.1.1 Digest and distil a reagent blank with each batch of assays, using the distillation apparatus.
- 6.4.1.2 Weigh 1.00 g of finely ground (<0.5 mm), air-dry soil into a 100 mL Kjeldahl digestion flask.
- 6.4.1.3 Include a reagent blank and a reference sample throughout the procedure.
- 6.4.1.4 Add 2.0 mL deionised water and, after swirling for a few minutes, allow to stand for 30 min.
- 6.4.1.5 Add two catalyst tablets (or 2.2 g catalyst mixture) and 6.5 mL of digest acid (conc. H_2SO_4).
- 6.4.1.6 Heat flasks cautiously in a fume cupboard or with an attached fume extractor until water is lost and frothing has ceased.
- 6.4.1.7 Increase heat so that H_2SO_4 condenses about one-third way up the neck of the digestion flasks. Continue boiling for 2 h after the soil has become bleached.
- 6.4.1.8 On completion of digestion, cool, then add slowly and with swirling, about 30 mL deionised water to prevent solidification of the digest solution on complete cooling.
- 6.4.1.9 There is no requirement to dilute to a known volume.

6.4.2 DISTILLATION AND TITRATION

- 6.4.2.1 Prepare the distillation apparatus by adding 10.0 mL of 2% H_3BO_3 to each 100 mL borosilicate beaker or flask.
- 6.4.2.2 Place the beaker under the condenser outlet so the end of the condenser is just below the surface of the H_3BO_3 solution.
- 6.4.2.3 Carefully add $\approx 25\text{--}30$ mL 60% NaOH Solution to each digestion flask and quickly attach the flask to the distillation unit; the solution should be distinctly alkaline, commonly indicated by the formation of a brown precipitate of ferric hydroxide – $(\text{Fe}(\text{OH})_3)$.
- 6.4.2.4 Distil for 5 min at a distillation rate of ≈ 8 mL/min until at least 30 mL are collected.
- 6.4.2.5 Lower the beaker so the condenser tip is above the solution and rinse the end of the condenser with deionised water.
- 6.4.2.6 Remove flasks and stop distillation. Temperature of the distillate should not exceed 40°C to prevent loss of N as NH_3 .
- 6.4.2.7 Add 5–6 drops of the mixed indicator and titrate the distillate with standardised 0.01 M HCl. The end point is indicated by a colour change from pale green to faint pink.

If preferred, the end point of the titration with 0.01 M HCl can be determined potentiometrically to pH 5.0, although the ionic strength of individual solutions can affect this pH slightly.

Notes:

1. The K_2SO_4 in the catalyst allows the digestion temperature to be increased to an optimal value between 380°C and 400°C. Temperatures below 360°C produce incomplete digestions and above 420°C losses occur. Boil until the digestion mixture becomes clear and then continue digestion for at least ten minutes, taking care that the acid is not completely consumed. The boiling time may be different and depends on the characteristics of the sample, but the solution must be clear at the end of the boiling. For most soils, a digestion time of 30-60 minutes is enough.
2. Each batch of 20-40 samples should contain at least two reagent blanks (no soil), and one or more reference samples.
3. If you use traditional digestion equipment and it is necessary, you can add glass beads or some pumice granules to reduce foaming. Boil the mixture gently so that the H_2SO_4 condenses in approximately the lower third of the tube.

6.5 CALCULATION OF RESULTS

$$\text{Nitrogen (\%)} = (T_2 - T_1) \times M \times 0.014 \times 100/W \times 50/v \times MF$$

where T_2 = sample titre (ml)

T_1 = blank titre (ml)

M = molarity of HCl

0.014 = weight of 1 mM nitrogen (g)

W = sample weight (g)

v = aliquot of sample taken (ml)

MF = moisture factor

For a 1.0 g sample, 0.01 M HCl on the total digest:

$$\text{Nitrogen (\%)} = (T_2 - T_1) \times 0.014 \times MF \text{ (Report results to two decimal places).}$$

Interpretation of results

Interpretation of total N measurements is difficult since only a very small percentage of total N is available to plants, and the N availability (N mineralisation) in soil is governed by many factors, e.g. quality of soil organic matter, edapho-climatic factors, etc. In the Pacific Islands, it is even more difficult as critical N values of Pacific Island soils are not yet determined. However, the following are some broad ratings of total N measurements (Blakemore et al. 1987).

Table 4. Ratings for total N (from Blakemore et al. 1987).

Rating	Total N (%)
Very high	> 1.0
High	0.6–1.0
Medium	0.3–0.6
Low	0.1–0.3
Very low	< 0.1

7.0 Olsen-available phosphorus (Method 9C1)

7.1 INTRODUCTION

Phosphorus (P) availability is a limiting factor for plant production in many agricultural soils. This is especially true in the highly weathered soils of the humid tropics. In the regions of the world without a history of use of P fertilisers, P deficiency is common. In the South Pacific, a large portion of applied P may be fixed to Fe and Al in volcanic soils as well as to Ca in atolls. The literature shows that 50–90% of applied P can be fixed in some Samoan soils (Asghar 1988). These facts make sound P management an imperative, especially in situations where funds for fertiliser purchases are limited, as in tropical smallholder agriculture. Because of generally low P concentrations in mulch materials, low atmospheric inputs, and low release by mineral weathering, adequate applications of P fertiliser are necessary in intensive agriculture to ensure economic and ecological sustainability.

In contrast to N, soil P is almost entirely derived from primary minerals, mainly apatite. Inorganic P is present in soil solution as phosphate ions ($\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$). It adsorbs on clay mineral surfaces by exchange and by specific bonding or precipitates with Ca and Al/Fe depending on the soil reaction. The pH for optimum P availability ranges from 5.5 to 6.5 when determined in water.

Numerous methods for determining available P are employed. Of these methods, the Olsen available P by the NaHCO_3 extraction method (developed by Olsen et al. in 1954 and later modified by Olsen and Sommers in 1987) correlated well with crop responses in South Pacific soils and is widely used in regional laboratories. This method is based on extraction (30 minutes) of air-dry soil with extracting solution (0.5 M Na_2CO_3) at a soil/suspension ratio of 1:20, adjusted to pH 8.5 with NaOH.

7.2 REAGENTS

- 7.2.1 EXTRACTING REAGENT (0.5 M NaHCO_3). Dissolve 42.0 g sodium hydrogen carbonate (NaHCO_3) in about 980 mL water. Adjust pH to 8.5 by adding dropwise approximately 10 M sodium hydroxide (40 g NaOH dissolved in 100 mL water). Must be made up immediately before use, that is, on the day of use.
- 7.2.2 SULFURIC ACID, 5 M. Carefully add 683 mL conc. H_2SO_4 to 2 L distilled or deionised water, cool, and make up to 2.5 L.
- 7.2.3 SULFURIC ACID, 1 M. Carefully add 27.3 mL conc. H_2SO_4 to distilled or deionised water, cool, and make up to 500 mL.
- 7.2.4 SULPHURIC ACID, 0.1 M. Dilute 100 mL 1 M H_2SO_4 to 1 L with distilled or deionised water.
- 7.2.5 MURPHY AND RILEY REAGENT A. Dissolve 12.0 g ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ in 250 mL distilled or deionised water. The rate of solution may be increased by heating, but do not heat above 60°C. Separately dissolve 0.2908 g potassium antimony tartrate ($\text{KSbOC}_4\text{H}_4\text{O}_6$) in 100 mL distilled water. Add both solutions, while stirring, to 1 L of cool 2.5 M H_2SO_4 (139 mL 18 M H_2SO_4 to 1 L with distilled or deionised water). Mix well, make to 2 L with distilled or deionised

water, and store in dark bottles. This reagent remains stable for a long time if stored in borosilicate glass in a dark and cool place.

- 7.2.6 MURPHY AND RILEY REAGENT B. Dissolve 1.056 g *l*-ascorbic acid ($C_6H_8O_6$) in 200 mL of reagent A and mix. Prepare as required on day of use as this reagent does not keep for more than 24 h.
- 7.2.7 p-NITROPHENOL INDICATOR. Dissolve 0.5 g p-nitrophenol ($NO_2C_6H_4OH$) in 25 mL deionised water.

7.3 STANDARDS

- 7.3.1 Phosphorus Primary Standard, STOCK SOLUTION (200 mg/L P). Dissolve 0.4394 g potassium dihydrogen orthophosphate, KH_2PO_4 (dried at 130°C for 2 h) in 0.5 M $NaHCO_3$ extracting solution and make to 500 mL. Add 2 drops of chloroform to suppress biological activity. Store solution in borosilicate glass in a dark and cool place, where it should remain stable for at least 6 months.
- 7.3.2 Phosphorus Secondary Standard, WORKING STOCK (20 mg/L P). Pipette 20 mL aliquot of stock solution (200 mg/L P) into a 200 mL volumetric flask and make to volume with 0.5 M $NaHCO_3$. This should be freshly prepared each time.
- 7.3.3 WORKING STANDARDS. Pipette 0, 2, 5, 10, 15 and 20 mL of the working stock solution (20 mg/L P) into 100 mL volumetric flasks and make to volume with 0.5 M $NaHCO_3$ for preparing 0, 0.4, 1, 2, 3 and 4 mg/L P standard solution.

7.4 PROCEDURE

7.4.1 EXTRACTION

- 7.4.1.1 Weigh 1.00 g soil (air-dry, <2 mm) into a screw cap 50 mL propylene centrifuge tube.
- 7.4.1.2 Add 20 mL extracting reagent. As the amount of phosphorus extracted is time dependent, it is important that the addition of reagents and later filtering is done without delay.
- 7.4.1.3 Include two reagent blanks and a reference sample throughout the procedure.
- 7.4.1.4 Shake for 30 min using an end-over-end shaker at about 50 rpm.
- 7.4.1.5 Filter through a No. 42 filter paper, into tubes or vials.
- 7.4.1.6 Cap and store in a refrigerator if not read immediately.

7.4.2 DETERMINATION

- 7.4.2.1 Pipette 4 mL sample filtrates or Standard Solutions into 25 mL stoppered tubes.
- 7.4.2.2 Add 1 drop of p-nitrophenol indicator and 10 mL 0.1 M H_2SO_4 to neutralise the extracting solution at pH 5.

7.4.2.3 Add 4 mL Murphy and Riley Reagent B, stopper and mix well.

7.4.2.4 After 30 min, measure absorbance of samples, standard and a reagent blank on the spectrophotometer at 882 nm against deionised water as a reference (Rayment and Lyons, 2011). Another, less sensitive peak at 660 nm can also be used if the spectrophotometer cannot be used at 882 nm. Solutions are stable for 24h.

7.5 CALCULATION

Prepare a standard curve of mg/L P against absorbance.

Read off unknowns as mg/L P.

$$\text{Olsen P (mg/kg)} = (a - b) \times 20 \times \text{MF}$$

where: a = P in sample solution (mg/L)

b = P in blank solution (mg/L)

20 = extraction ratio

MF = moisture factor.

Report results: use a decimal place when results range between 1 and 10 mg/kg, and no decimal places if results are > 10 mg/kg.

Interpretation of results

Critical P values of Pacific Island soils have not yet been determined. Therefore it is difficult to interpret analytical results for these soils. However, the following are some broad ratings of Olsen P measurements (Blakemore et al. 1987). Based on Table 5, it is possible to interpret the data for fertiliser recommendation. For example, if your available P values fall under high rating, then fertiliser response is unlikely; if medium, a response is probable; and if low, a response is most likely.

Table 5. Ratings for Olsen P (from Blakemore et al. 1987).

Rating	Olsen P (mg/kg)
Very high	>50
High	30–50
Medium	20–30
Low	10–20

Very low	<10
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8.0 Exchangeable bases (Ca, Mg, K and Na) (Method 15A1)

8.1 INTRODUCTION

The cations potassium (K), magnesium (Mg) and calcium (Ca) occur in several forms in soil that differ in their availability to plants. The most readily available fraction for plants is the fraction in the soil solution, followed by the exchangeable fraction, which replenishes the soil solution if the nutrients are removed by either plant uptake or leaching.

Exchangeable cations are those that can be exchanged by a cation of an added salt solution. The exchangeable cations Ca^{2+} , Mg^{2+} , K^{+} and Na^{+} , which are often called the exchangeable bases, commonly occur in soils in quantities decreasing in the order listed. In most agricultural soils Ca^{2+} occurs in larger quantities than all the other bases combined. In some soils formed from serpentine, Mg^{2+} may be higher than Ca^{2+} . This tendency is also found in acid subsoils on older landscapes. Potassium is usually the third most abundant cation, but this varies because of soil parent material and agricultural treatments. Sodium in most soils is very low, and in many humid region soils is only found in traces.

Determination of the exchangeable bases is useful because these are the elements forms that are considered to be plant available by exchange with hydrogen ions from the exudates of root hairs and soil micro-organisms. Therefore, the amount of exchangeable bases present are important for plant growth. In addition to the individual levels of cations, their ratios are also important in crop growth.

Numerous soil test methods are used for assessing the availability of these nutrients to plants. The replacement of exchangeable cations using the NH_4OAc method (buffered to pH 7) is followed in Pacific Island laboratories. However, with the recent adoption of inductively coupled plasma optical emission spectroscopy (ICP-OES) in routine soil-testing laboratories, soil extraction with 1.0 M ammonium chloride (NH_4Cl) has become an alternative due to the possibility of determining all exchangeable elements in one run (Ca, Mg, K, Mn, Na, and Al) (Gianello and Amorim 2015).

This method includes three procedures for the extraction of the exchangeable bases. The shaking extraction is used if exchangeable bases only are needed. The shaking extraction for exchangeable bases is adapted from the method described by Daly et al. (1984).

Solutions are diluted prior to measurement of bases by an Atomic Absorption Spectrometer (AAS), to reduce the salt level and thus prevent nebuliser blockage. Caesium (Cs) is added to eliminate ionisation interference in the determination of K and Na, and strontium (Sr) is added to prevent chemical interference in the determination of Ca and Mg.

8.2 REAGENTS

8.2.1 EXTRACTING SOLUTION, 1 M AMMONIUM CHLORIDE at pH 7.0. Dissolve 535 g ammonium chloride (NH_4Cl – low in Ca, Mg, Na and K impurities) in deionised water and dilute to 1 L. Adjust to pH 7.0 by adding ammonium hydroxide (NH_4OH). Wash the electrodes of the pH meter thoroughly before placing them in the extracting solution, otherwise K^{+} salts from the calomel electrode may cause contamination.

Make the volume to 10 L with deionised water and store in sealed containers. Plastic containers are preferred though borosilicate glassware may be substituted. Soda-glass should not be used.

- 8.2.2 5 M AMMONIUM CHLORIDE AT pH 7.0. Dissolve 267.5 g NH_4Cl (identical to that used in the extracting solution) and dilute to 900 mL. Adjust to pH 7.0 as described for the extracting solution and make to 1.0 L.

- 8.2.3 STRONTIUM CHLORIDE SOLUTIONS (only required if using an AAS analytical finish).

Sr Stock Solution 1 L contains 8.33 g of Sr. Dissolve 25.35 g strontium chloride ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$) and dilute to 1 L with deionised water. Store in a plastic or borosilicate bottle.

Sr Working Solution 1 L contains 1.67 g of Sr. Dilute 200 mL Sr stock solution to 1 L with deionised water. Store in a plastic or borosilicate bottle.

Sr Diluting Solution for Ca and Mg Analysis by AAS 1 L contains 1.50 g of Sr. Add 180 mL Sr stock solution to 100 mL NH_4Cl extracting solution (identical to that used for soil extractions) and dilute to 1 L with deionised water.

- 8.2.4 WETTING AGENT – BRIJ 35. Shake 30 g of polyoxyethylene 23 lauryl ether (Brij 35) with 20 mL iso-propyl alcohol [propane-2-ol, $(\text{CH}_3)_2\text{-CH-OH}$] until dissolved; several hours may be required. Make to 100 mL with deionised water.

- 8.2.5 LITHIUM CHLORIDE FOR AUTOMATED Na^+ and K^+ . Dissolve 0.11 g lithium chloride (LiCl), add 1 mL Brij 35 Wetting Agent, and make to 1 L with deionised water.

- 8.2.6 MIXED Ca AND Mg PRIMARY STANDARD. 1 L contains 100 mmol_c of Ca and Mg. Use certified commercial standard concentrated, or dry calcium carbonate (CaCO_3 , primary standard grade) by heating at 110°C to constant weight. Dry magnesium oxide (MgO , heavy) by heating in an electric muffle furnace at 600–700°C for 2 h. Cool and store the chemicals in a desiccator without desiccant.

Weigh 2.0152 g MgO and 5.0045 g CaCO_3 and wash into a 1 L conical flask with about 50 mL deionised water. Add 240 mL 1 M HCl and boil until all CO_2 is expelled. Cover and allow to cool, then transfer quantitatively to a 1 L volumetric flask. Dilute to volume with CO_2 -free (boiled) deionised water and mix well. Transfer to a clean plastic bottle. Should MgO not assay at 100% purity, adjust the weight according to the assay obtained.

- 8.2.7 MIXED Na AND K PRIMARY STANDARD. 1 L contains 50 mmol_c of Na and 12.5 mmol_c of K. Use certified commercial standard concentrates, or dry sodium chloride (NaCl) at 105°C for 2 h and potassium chloride (KCl) for 2 days at 115–120°C. When dry, cool and store in a desiccator without a desiccant.

Weigh 2.9221 g NaCl and 0.9319 g KCl and dissolve separately with deionised water. Transfer quantitatively to a 1 L volumetric flask and make to 1.0 L with deionised water. Store in a clean plastic bottle.

- 8.2.8 MIXED Ca AND Mg SECONDARY STANDARD. 1 L contains 10 mmol_c of Ca and Mg. Take 50 mL of Mixed Ca and Mg Primary Standard and dilute to 500 mL in a volumetric flask with CO_2 -

free (boiled) deionised water. This solution should be freshly prepared each time working standards are required.

8.2.9 MIXED Na AND K SECONDARY STANDARD. 1 L contains 5 mmol_c of Na and 1.25 mmol_c of K.

Take 50 mL of Mixed Na and K Primary Standard and dilute to 500 mL in a volumetric flask with CO₂-free deionised water. This solution should be freshly prepared each time working standards are required.

8.2.10 MIXING WORKING STANDARDS. Dispense mixed Ca and Mg secondary and primary Standards, as indicated in Table 6, and Mixed Na and K secondary and primary Standards as indicated in Table 7, into 500 mL volumetric flasks. Add 100 mL 5 M NH₄Cl to each and dilute to 500 mL with CO₂-free deionised water.

Table 6. Example of dilutions and concentrations for Ca and Mg working standards, 1 M NH₄Cl at pH 7.0

mL of mixed primary or secondary standard in 500 mL	Initial solution concentration (mmol _c Ca and Mg/ L)	Equivalent soil content (cmol _c /kg) of Ca and Mg, respectively, for 1:20 soil/extract ratio (final) following:	
		1 + 9 dilution ^a of samples and standards	1 + 49 dilution ^{b,c} of samples and standards; 1 + 9 dilution ^a of standards
Mixed Ca and Mg Secondary Standard (10 mmol _c Ca and Mg/L)			
2.5	0.05	0.1	0.5
5.0	0.10	0.2	1.0
7.5	0.15	0.3	1.5
12.5	0.25	0.5	2.5
25.0	0.50	1.0	5.0
50.0	1.00	2.0	10.0
Mixed Ca and Mg Primary Standard (100 mmol _c Ca and Mg/L)			
7.5	1.5	3.0	15.0
10.0	2.0	4.0	20.0
12.5	2.5	5.0	25.0
15.0	3.0	6.0	30.0
20.0	4.0	8.0	40.0
25.0	5.0	10.0	50.0

^a Dilute 1 part Working Standards and sample extracts with 9 parts Sr Working Solution if analysing by AAS. For ICPAES, substitute the Sr Working Solution with NH₄Cl Extracting Solution.

^b AAS analysis requires accurate 1:5 dilution of samples already diluted 1+9 with Sr Working Solution. Use Sr Diluting Solution for Ca and Mg Analysis by AAS (1 L contains 1.50 g Sr) as diluent. Working Standards already diluted 1+9 should not be further diluted.

^c For ICPAES analysis, substitute the Sr Working Solutions with NH₄Cl Extracting Solution.

Table 7. Example of dilutions and concentrations for Na and K working standards, 1 M NH₄Cl at pH 7.0

mL of mixed primary or secondary standard in 500 mL	Initial solution concentration (mmol _c /L)		Equivalent soil content (cmol _c /kg) for 1:20 soil/ extract ratio ^a	
	Na	K	Na	K
Mixed Na and K Secondary Standard (5 mmol _c Na/L and 1.25 mmol _c K/L)				
2.5	0.025	0.006	0.05	0.0125
7.5	0.075	0.019	0.15	0.038
12.5	0.125	0.031	0.2	0.063
25.0	0.25	0.063	0.5	0.125
50.0	0.50	0.125	1.0	0.25
Mixed Na and K Primary Standard (50 mmol _c Na/L and 12.5 mmol _c K/L)				
7.5	0.75	0.188	1.5	0.375
10.0	1.00	0.250	2.0	0.500
12.5	1.25	0.313	2.5	0.625
15.0	1.50	0.378	3.0	0.75
20.0	2.0	0.500	4.0	1.00
25.0	2.5	0.625	5.0	1.25

^a If necessary, dilute extracts of high concentration with 1 M NH₄Cl Extracting Solution to bring these within the optimum range of the instrument, and to maintain the same concentrations of NH₄Cl in standard and sample extracts.

8.3 PROCEDURE

8.3.1 EXTRACTION

8.3.1.1 Weigh 5.00 g of air-dried soil (< 2mm) into a 250 mL plastic extracting bottle and add 100 mL 1 M NH₄Cl at pH 7.0 Extracting Solution.

8.3.1.2 Include reagent blanks and reference samples throughout the procedure.

8.3.1.3 Secure with stopper and mechanically shake end-over-end at ≈25°C for 1 h.

8.3.1.4 Centrifuge or filter the extracts. If filtering, prepare Whatman No. 40 filter papers in 75 mm plastic funnels and place suitable clean, dry, receiving containers in position. Discard the first 10–20 mL of filtered extract then collect sufficient extract (30–50 mL) to determine all cations. If centrifuging, ensure centrifuge tubes are clean and dry.

8.3.1.5 Retain the clarified extracts for Ca²⁺, Mg²⁺, Na⁺ and K⁺ analyses. The batch should be sized to allow filtration and/or centrifugation to occur within 30 mins of mechanical shaking completion .

8.3.2 DETERMINATION OF Ca²⁺, Mg²⁺, Na⁺ and K⁺ by AAS

8.3.2.1 Follow the manufacturer's recommendation with respect to instrument parameters. An air-acetylene flame is usual for all elements. Preferred spectral lines are: Ca = 422.7 nm; Mg = 285.2

nm; Na = 589.0 nm; and K = 766.5 nm. Ca^{2+} and Mg^{2+} are measured on dilute extracts, whereas extracts of Na^+ and K^+ analysis are only diluted if over range.

8.3.2.2 A reagent blank should also be measured, and adjustments made as necessary.

8.3.2.3 For Ca^{2+} and Mg^{2+} , dilute each soil extract and each Ca and Mg Working Standard with Sr Working Solution as indicated in Table 6, noting that Sr is included to suppress interferences in measurement due to phosphate and other ions. Dilute samples only as indicated in Table 6 to maintain Ca^{2+} and Mg^{2+} concentrations if above the optimum range of the instrument. Mix well, then determine Ca^{2+} and Mg^{2+} concentrations directly.

For measurement of Na^+ and K^+ by AAS, use a selection of Working Standards (Table 7). Determine concentrations of Na^+ and K^+ in soil extracts directly from the instrument.

8.3.2.4 Settings and operation of the instrument should be checked periodically.

8.3.3 DETERMINATION OF Ca^{2+} , Mg^{2+} , Na^+ and K^+ by ICPAES

8.3.3.1 Set up and operate the ICPAES instrument as advised by the manufacturer. Suitable wavelengths are: Ca = 430.25 nm; Mg = 285.2 nm; Na = 588.96 nm; and K = 766.49 nm. However, this is dependent on the instrument and concentrations.

8.3.3.2 Calibrate the instrument using an appropriate range of Working Standard Solutions, guided by examples in Table 6 and 7. The 1:20 soil/extraction ratio can be factored into the calibration on the ICPAES.

8.4 CALCULATIONS

Report exchangeable Ca^{2+} , Mg^{2+} , Na^+ and K^+ (cmol_c/kg) on an oven-dry soil basis by multiplying by the moisture factor (MF). Report results to three decimal places.

Interpretation of results

Follow Table 8 for ratings and interpretation of data. Critical values of these elements for South Pacific Island soils have not yet been determined, but the following are broad ratings of Ca, Mg, K and Na measurements (Blakemore et al. 1987).

Table 8. Ratings for sum of bases, % base saturation and cation exchange properties (from Blakemore et al. 1987).

Cation exchange properties (NH ₄ OAc, pH 7)						
Rating	Σ Bases	BS	Ca	Mg	K	Na

	cmol(+)/kg	(%)	_____ cmol(+)/kg _____			
Very high	>25	80–100	>20	>7	>1.2	>2
High	15–25	60–80	10–20	3–7	0.8–1.2	0.7–2
Medium	7–15	40–60	5–10	1–3	0.5–0.8	0.3–0.7
Low	3–7	20–40	2–5	0.5–1	0.3–0.5	0.1–0.3
Very low	<3	<20	<2	<0.5	<0.3	<0.1

9.0 DTPA extractable Fe, Mn, Cu and Zn (Method 12A1)

9.1 INTRODUCTION

Testing for micronutrients for soil fertility assessment and heavy metals for resource condition assessment is increasing. Micronutrient contents in soil are tested to identify whether the soil is deficient of a specific micronutrient element, or whether it is present in a high concentration that is toxic to the plants. Toxicity of some micronutrients may develop in soil due to industrial pollution, mining or repeated use of crop fungicides.

An extracting solution containing a chelating agent such as diethylenetriaminepentaacetic acid (DTPA) removes micronutrient cations adsorbed on solid phases together with water-soluble constituents. Chelating agents are used as extractants to estimate the plant availability of trace elements in soil as they simulate the action of plant roots. With the DTPA method of Lindsay and Norvell (1978), although designed for slightly acidic or alkaline soils, there may be insufficient capacity in the chelating agent to extract all of the extractable iron (Fe) and manganese (Mn).

This method involves equilibration of air-dry soil for 2 hours with extracting solution at a soil/suspension ratio of 1:2 at pH 7.3. Buffering the extracting solution at pH 7.3 restricts dissolution of trace metals from soils of high pH.

Currently, measurement of Fe, Mn, copper (Cu) and zinc (Zn) by ICPAES is preferred, but AAS provides an acceptable alternative analytical finish of the Fe, Mn, Cu and Zn extracted by the DTPA reagent at pH 7.3.

9.2 REAGENTS

9.2.1 DTPA EXTRACTING SOLUTION. This solution is 0.005 M with respect to DTPA, 0.01 M to CaCl_2 and 0.10 M to triethanolamine (TEA). For 1.0 L of Extracting Solution, dissolve 1.97 g DTPA, 1.47 g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and 14.92 g triethanolamine [$\text{N}(\text{CH}_2\text{CH}_2\text{OH})_3$] separately in deionised water and combine. Add ≈ 6.8 g of 35% w/w HCl and dilute to ≈ 990 mL with deionised water. Check pH and adjust to 7.3 ± 0.05 with either diluted HCl or triethanolamine, then make volume to 1.0 L. Store in a Teflon or low-density polyethylene container not previously used to store any of the four metals under test. The solution will remain stable for at least 3 months if kept cool ($\approx 4^\circ\text{C}$) and away from direct sunlight.

9.3 STANDARDS

9.3.1 COPPER PRIMARY STANDARD.

1 mL contains 1 mg Cu (1000 ppm).

Use certified commercial standard concentrated copper (Cu) or clean a piece of copper foil then accurately weigh 1.000 g of the cleaned metal and place in a 1 L volumetric flask. Dissolve in 20 mL of 1+1 (v/v conc. $\text{HNO}_3:\text{H}_2\text{O}$) HNO_3 and dilute to volume with deionised water.

9.3.2 ZINC PRIMARY STANDARD. 1 mL contains 1 mg Zn (1000 ppm). Use certified commercial standard concentrated or clean a piece of Zn rod then accurately weigh 1.000 g of the cleaned metal and place in a 1 L volumetric flask. Dissolve in 20 mL 1+1 HCl and dilute to volume with deionised water.

9.3.3 MANGANESE PRIMARY STANDARD. 1 mL contains 5 mg of Mn (5000 ppm). Use certified commercial standard concentrated manganese (Mn) or weigh 6.8712 g anhydrous manganous sulfate (prepared by dehydrating manganese sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$) at 200°C for 4 h) into a 500 mL volumetric flask. Dissolve in a mixture of 200 mL water and 1 mL 18 M H_2SO_4 and make to volume with deionised water.

9.3.4 IRON PRIMARY STANDARD.

1 mL contains 5 mg Fe (5000 ppm).

Use certified commercial standard concentrated iron (Fe) or weigh 17.5538 g ammonium ferrous sulfate [$(\text{NH}_4)_2\text{SO}_4\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$] and transfer to a 500 mL volumetric flask. Dissolve in a mixture of 200 mL water and 1 mL 18 M H_2SO_4 and make to volume with deionised water.

9.3.5 MIXED 'LOW STRENGTH' SECONDARY STANDARD. Take 10.0 mL copper (Cu) primary standard, 10.0 mL Zn primary standard, 20.0 mL Mn primary standard and 20.0 mL Fe primary standard and dilute with deionised water to 1 L. This solution contains 10 mg/L of both Cu and Zn and 100 mg/L of both Mn and Fe.

9.3.6 MIXED 'HIGH STRENGTH' SECONDARY STANDARD. Take 40.0 mL Cu primary standard, 40.0 mL Zn primary standard, 200 mL Mn primary standard and 200 mL Fe primary standard and dilute with deionised water to 1.0 L. This solution contains 40 mg/L of both Cu and Zn and 1000 mg/L of both Mn and Fe.

9.3.7 MIXED WORKING STANDARDS. The aliquots of freshly prepared 'low' and 'high' strength secondary standards and details of the resulting concentrations are given in Table 9. Add 83 mL triple strength DTPA extracting solution (45 g triethanolamine, 5.91 g DTPA, 4.41 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 20.65 g HCl and make to 1 L with deionised water). Store in black polyethylene bottles or in the dark or in standard polyethylene or Teflon bottles. Actual solution concentrations and equivalent soil contents for a 1:2 soil/extract ratio are given in Tables 9 and 10.

Table 9. Volumes of 'low' strength secondary standard and resulting concentrations of 'low range' mixed working standards for DTPA-extractable Cu, Zn, Mn and Fe.

mL of 'low' strength secondary	Actual solution concentration (mg/L)		Equivalent soil content (mg/kg) for a 1:2 soil/extract	
	Cu and Zn	Mn and Fe	Cu and Zn	Mn and Fe

standard in 100 mL				
0	0	0	0	0
1.0	0.1	1	0.2	2
2.0	0.2	2	0.4	4
3.0	0.3	3	0.6	6
4.0	0.4	4	0.8	8
5.0	0.5	5	1.0	10
6.0	0.6	6	1.2	12
8.0	0.8	8	1.6	16
10.0	1.0	10	2.0	20
15.0	1.5	15	3.0	30
20.0	2.0	20	4.0	40

Table 10. Volumes of 'high' strength secondary standard and resulting concentrations of 'high range' mixed working standards for DTPA-extractable Cu, Zn, Mn and Fe.

mL of 'high' strength secondary standard in 100 mL	Actual solution concentration (mg/L)		Equivalent soil content (mg/kg) for a 1:2 soil/extract	
	Cu and Zn	Mn and Fe	Cu and Zn	Mn and Fe
0	0	0	0	0
1.0	0.4	10	0.8	20
2.0	0.8	20	1.6	40
4.0	1.6	40	3.2	80
6.0	2.4	60	4.8	120
8.0	3.2	80	6.4	160
10.0	4.0	100	8.0	200
12.0	4.8	120	9.6	240
14.0	5.6	140	11.2	280
16.0	6.4	160	12.8	320
18.0	7.2	180	14.4	360
20.0	8.0	200	16.0	400
24.0	9.6	240	19.2	480

Table 11. Typical instrument parameters for analysis of DTPA soil extracts by AAS.

Element	Range of standards (mg/L)	Instrument sensitivity	Spectral line (nm)	Approx. range for calibration curves (mg/L)
Cu	0.1–2.0	High	324.7	0.1–2.0
	0.4–7.2	Low	324.7	0.4–5.0
Zn	0.1–2.0	High	213.8	0.1–1.5
	0.4–7.2	Low	213.8	0.4–4.0
Mn	1.0–10.0	High	279.4	1.0–4.0

	10.0–18.0	Low	403.0	10.0–80
Fe	1.0–10.0	High	248.3	1.0–6.0
	10.0–18.0	Low	372.0	10.0–60

Additional notes:

1. Cu and Zn should be first polished with steel wool to remove any surface coating. Immerse a piece of the metal in the appropriate acid – (1+1) HNO₃ for Cu, (1+1) HCl for Zn – until a clean surface is visible. Wash with deionised water and glass-distilled acetone. Dry at room temperature and weigh immediately. Alternatively, commercial primary Standard Solutions may be used for the four elements.
2. With AAS, atomic spectral lines vary with element and with concentration range standards selected for use. The guidelines are given in Table 11.
3. Preferred spectral lines for ICPAES are typically 324.754, 213.856, 257.610 and 259.940 nm for Cu, Zn, Mn and Fe, respectively. No background corrections are necessary when these wavelengths are used over concentration ranges of 0–10 mg/L for Cu and Zn and 0–240 mg/L for Mn and Fe.

9.4 PROCEDURE

9.4.1 EXTRACTION

- 9.4.1.1 Weigh 25.0 g soil (air-dry, < 2mm) and place into a 100 or 250 mL polyethylene bottle.
- 9.4.1.2 A reagent blank with no soil and a reference sample should be included with each batch of samples.
- 9.4.1.3 Add 50 mL of DTPA extracting solution, stopper, and mechanically shake end-over-end continuously for 2 h at 25°C.
- 9.4.1.4 Filter (No. 2 Whatman filter paper) or centrifuge the extracts without delay, discarding the first portion and retaining the particle-free extracts for analysis.

9.4.2 DETERMINATION

- 9.4.2.1 Measure metal concentrations in these filtrates by ICPAES or AAS as soon as possible to avoid microbial growth and/or chemical changes.
- 9.4.2.2 Use an appropriate selection of working standards and determine concentrations of each element (mg/kg) from the appropriate calibration curve, after adjusting for any significant reagent blank. It is important to follow the manufacturer's recommendations with respect to instrument parameters and wavelength selections.

9.5 CALCULATIONS

Report each element (Cu, Zn, Mn, Fe; mg/kg) on an air-dry basis by multiplying by the moisture factor (MF). Report results to three decimal places.

Interpretation of results

Follow Table 12 for ratings and interpretation of data. Values are from Lindsay and Norvell (1978) and were derived on Mollisols in the USA. Calibrations have not been done on tropical soils in the Pacific so these figures should be used with caution.

Table 12. Ratings of micronutrients (Cu, Zn, Mn and Fe).

Rating	Fe	Mn	Cu	Zn
	(mg/kg)			
Deficient	<2.5	–	–	<0.8
Low	2.5–4.5	<10	<0.2	0.8–1.2
Adequate	>4.5	>1.0	>0.2	>1.2

10.0 Calcium chloride extractable boron (Method 12C1)

10.1 INTRODUCTION

Hot water extraction has commonly been used to estimate the availability of boron (B) in soils. The method was modified by Parker and Gardner (1981) to use hot 0.02 M calcium chloride (CaCl_2) as the extractant. This modification minimises analytical problems caused by organic matter extraction and clay dispersion. However, some soil extracts are still coloured by organic matter as most of the Pacific Islands' volcanic soils are very rich in organic matter, which causes interference with the colorimetric method. Activated charcoal needs to be added to absorb the extracted organic matter, but because it can absorb or release boron, it needs to be added to the standards as well as the samples.

The concentration in the extracts is determined by Gaines and Mitchell's (1979) version of the azomethine-H method.

Boron is a ready contaminant in the laboratory, and is also a constituent of laboratory borosilicate glassware, so care must be taken to prevent sample contamination. In practice this means acid washing glassware and plastic prior to use and using glassware for short-term storage only.

10.2 REAGENTS

- 10.2.1 0.01 M CALCIUM CHLORIDE EXTRACTING SOLUTION. Dissolve 1.47 g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in water and make to 1.0 L. Store in B-free glassware or a polyethylene or Teflon container.
- 10.2.2 ACTIVATED CHARCOAL. Add 1.0 L CaCl_2 Extracting Solution to 500 g of activated charcoal and reflux for 10 min. Filter CaCl_2 Extracting Solution while hot ($\approx 80^\circ\text{C}$). Dry the charcoal in an oven at 60°C .
- 10.2.3 0.025 M EDTA SOLUTIONS. Dissolve 4.65 g of disodium EDTA $\{[\text{CH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{COONa}]_2 \cdot 2\text{H}_2\text{O}\}$ in 200 mL deionised water and make to 500 mL. Add 1 mL Brij 35 Wetting Agent and mix thoroughly.
- 10.2.4 BUFFER SOLUTION. Dissolve 250 g ammonium acetate ($\text{CH}_3\text{COONH}_4$) in 500 mL deionised water and adjust solution pH to 5.5 by slowly adding ≈ 100 mL glacial acetic acid (CH_3COOH) with constant stirring. Add 0.5 mL Brij 35 Wetting Agent and remix.
- 10.2.5 AZOMETHINE-H SOLUTION. Azomethine-H is available commercially or may be prepared as described by Shanina et al. (1967). Dissolve 0.5 g of azomethine-H in ≈ 50 mL of deionised water. Add 1.0 g *l*-ascorbic acid ($\text{C}_6\text{H}_6\text{O}_6$) and warm gently (30°C) to obtain a clear solution. Cool and dilute to 100 mL. The solution remains stable for a week if stored at $\approx 4^\circ\text{C}$.
- 10.2.6 BORON PRIMARY STANDARD. 1 L contains 250 mg of B (250 ppm).

Use commercial primary Standard Solutions or dissolve 1.430 g AR boric acid (H_3BO_3 ; dried to constant weight in a desiccator over anhydrous CaCl_2)¹ in 0.01 M HCl and make to 1 L in a volumetric flask with 0.1 M HCl. Store in polyethylene or Teflon bottle.

10.2.7 BORON SECONDARY STANDARDS.

1 L contains 20 mg of B (20 ppm).

Pipette 40 mL B primary standard into a volumetric flask and make volume to 500 mL with 0.01 M CaCl_2 Extracting Solution. Store in a Teflon bottle; shelf life is about 4 weeks.

10.2.8 BORON WORKING STANDARDS.

Pipette 1.25, 2.5, 5, 10, 15, 20 and 25 mL B Secondary standard solution into separate 500 mL volumetric flasks. Dilute to volume with 0.01 M CaCl_2 Extracting Solution. These solutions contain 0.05–1.0 mg B/L. For a 1:2 soil/solution ratio, equal volumes of standards contain concentrations of B equivalent to 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mg B/kg of soil.

Working standards equivalent to 50 mg B/kg may be required when B toxicity in soil is suspected. The extracting solution serves as a blank.

10.3 PROCEDURE

10.3.1 EXTRACTION

All glass and plasticware must be washed with dilute 1+4 (1 V : 4 V; conc. HCl : H_2O) HCl followed by deionised water before use. Filter papers should be checked that they are free from B contamination. If contaminated, pre-treat by washing with hot ($\approx 80^\circ\text{C}$) CaCl_2 Extracting Solution.

10.3.1.1 Add 10 g of air-dried soil (< 2 mm) and 20 mL of 0.01 M CaCl_2 extracting solution into a B-free flat bottom flask or bottle (150 mL). Record the weight of each flask plus the fresh sample and extracting solution.

10.3.1.2 A reagent blank with no soil and a reference sample should be included with each batch of samples.

10.3.1.3 Insert a small plastic funnel in the neck of the flask and quickly bring to boil, then gently reflux for 10 min.

10.3.1.4 Remove the funnel and immediately bring the flask + sample back to its original weight with hot ($> 80^\circ\text{C}$) deionised water.

¹ If heated, H_3BO_3 gradually loses water to initially metaboric acid (H_3BO_2) and finally the anhydrous oxide (B_2O_3). Therefore, oven heating must not be used to dry H_3BO_3 .

- 10.3.1.5 Quickly filter the extraction through Whatman No. 40 filter paper into a polyethylene container.
- 10.3.1.6 If the hot ($\approx 80^{\circ}\text{C}$) extract is not colourless following soil extraction, add approximately 0.1 g activated charcoal to the filtered extract, shake by hand for about 5 mins, and filter through Whatman No. 42 paper. Repeat treatment with activated charcoal if necessary.
- 10.3.1.7 Cool then transfer a known suitable aliquot (e.g. 5 mL) into a polyethylene container.
- 10.3.1.8 Add 2.0 mL of 0.025 M EDTA solution and 2.0 mL of buffer solution. Mix flask contents well then add 2.0 mL Azomethine-H solution and shake well.
- 10.3.1.9 Set aside for 2 h for colour development, away from sunlight. Treat the same aliquot of each working standard in a similar manner.

10.3.2 READING

- 10.3.2.1 Read absorbance of both standards and assays with a spectrophotometer at 420 nm, using identical times for standards and unknowns. The colour is stable for 4 hours.
- 10.3.2.2 Construct a calibration curve (or a regression equation) and determine concentrations of B in soil extracts. Make allowances for any significant reagent calibration blank.

10.4 CALCULATION

Prepare a standard curve of mg/L B against absorbance.

Read off unknowns as mg/L B.

$$\text{Available B mg/kg} = (a - b) \times 20/W \times \text{MF}$$

where: $a = \text{B in sample solution (mg/L)}$

$b = \text{B in blank solution (mg/L)}$

20 = volume of extractant

W = sample weight (g)

MF = moisture factor.

Interpretation of results

Follow Table 13 for ratings and interpretation of data. Calibrations have not been done on tropical soils in the Pacific so these figures should be used with caution.

Table 13. Ratings of boron in soil (from Reisenauer et al. 1973).

	B (mg/kg)
Deficient	<1.0
Normal/adequate	1.0–5.0
Toxic	>5.0

11.0 Calcium phosphate-extractable sulphur (Method 10B1)

11.1 INTRODUCTION

In the Pacific Islands, the sea breeze is a source of sulphur (S). Therefore, S deficiency is not very common in Pacific Island soils, consequently laboratories in PICs do not usually determine S. The calcium phosphate-extractable sulphur method given here is not commonly used in Pacific Islands laboratories, however, it is included because a similar method was also described in Daly and Waniqolo (1993a).

Two different methods utilise calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) for the extraction of soil S, one with and other without activated charcoal. Here, the method that does not use activated charcoal will be described. In this method Sulfate S is extracted in absence of charcoal from soil by 0.01 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$ at pH 4 using soil solution ratio of 1:5 and an extraction time of 17 h at 25°C. This extracting solution contains sufficient phosphate ions to displaced adsorbed S from all soils.

The extracted S is determined by the method of Johnson and Nishita (1952) through manual distillation. Sulphate ions are reduced to hydrogen sulfide (H_2S), and the evolved gas absorbed in a solution of Zn^{2+} and sodium acetate. The sulfide anion is then allowed to react with p-aminodimethylaniline sulfate and H^+ to form methylene blue in the presence of Fe^{3+} , permitting the colorimetric measurement of Phosphate-extractable S.

11.2 REAGENTS

11.2.1 EXTRACTING SOLUTION – 0.01 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$ at pH 4.

To prepare 50 L of extracting solution:

(a) Calcine approximately 50 g calcium carbonate (CaCO_3) by placing a sufficient quantity into a furnace at 800°C. Raise the temperature to 1000°C and maintain temperature for 45 mins. Cool in a desiccant-free desiccator then add 60 g calcined calcium carbonate or calcium oxide (CaO) to 30 L deionised water, use a stopper, shake well, and allow the $\text{Ca}(\text{OH})_2$ solution formed to stand overnight.

(b) Weigh 116.82 g phosphoric acid (H_3PO_4) and transfer to a container holding not more than 15 L deionised water.

(c) Add ≈ 22 L suspension-free $\text{Ca}(\text{OH})_2$ solution (a) in 2 L aliquots with constant stirring to the dilute H_3PO_4 solution (b). Adjust pH to 4.0 with either H_3PO_4 or $\text{Ca}(\text{OH})_2$ solution. Make to volume with deionised water.²

² The theoretical quantity of saturated $\text{Ca}(\text{OH})_2$ solution required for 50 L of 0.01 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extracting solution is 24 L, but this varies with temperature. The solubility of $\text{Ca}(\text{OH})_2$ increases with decreasing solution temperature.

11.2.2 REDUCING AGENT. Mix together in a suitable 2 L refluxing flask:

600 mL 55% (w/v) hydriodic acid (HI)

150 mL sg. 1.13 hypophosphorous acid (H_3PO_2) and

300 mL 90% (v/v) formic acid (HCOOH).

Boil carefully below 117°C under reflux in a fume cupboard with a stream of N_2 gas bubbling throughout the solution. Continue boiling for about 10 min after the solution has cleared. (If boiled above 117°C , highly poisonous phosphine gas (PH_3) may form.)

When cool, disconnect N_2 and transfer the reducing agent to a dark storage bottle. If protected from light and atmosphere, the reagent remains stable for 1–2 months. Used reducing agent may be regenerated in a similar way up to a total of three times, provided its $\text{NO}_3\text{-N}$ content remains low.

11.2.3 ADSORBING SOLUTION. Dissolve 50.0 g zinc acetate dihydrate [$(\text{CH}_3\text{COO})_2\text{Zn}\cdot 2\text{H}_2\text{O}$] and 12.5 g sodium acetate trihydrate ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$) in deionised water and make to 1 L. Filter (Whatman No. 42 paper) before use.

11.2.4 AMINODIMETHYLANALINE SOLUTION. Dissolve 1.0 g N,N-dimethyl-*p*-phenylene diamine sulfate [$\text{NH}_2\text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2\cdot \text{H}_2\text{SO}_4$] in 70 mL deionised water. Add carefully 200 mL 18 M H_2SO_4 in small portions, cooling and mixing between additions. Cool and make to 1 L in a volumetric flask. Avoid free base formation during preparation, as this chemical is a mild vesicant (blisters or burns body tissues by contact with skin or following inhalation).

11.2.5 FERRIC IRON SOLUTION. To 125.0 g ferric ammonium sulfate [$\text{FeNH}_4(\text{SO}_4)_2\cdot 12\text{H}_2\text{O}$] add 25 mL 18 M H_2SO_4 and 975 mL deionised water. Invert several times to dissolve.

11.2.6 GAS WASHING SOLUTION. Dissolve 10.0 g sodium dihydrogen orthophosphate ($\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$) and 10.0 g pyrogallol [$\text{C}_6\text{H}_3(\text{OH})_3$] in 100 mL deionised water with aid of a stream of N_2 gas bubbling through the solution. Keep well sealed prior to use. Make fresh weekly or when the solution in the gas washing column is highly discoloured.

11.2.7 SULFATE PRIMARY STANDARD.

1 mL contains 1 mg of S (1000 ppm).

Use commercial primary Standard Solutions, or dissolve 5.4343 g potassium sulfate (K_2SO_4 ; previously dried at 105°C for 4 h) in deionised water and make to 1 L in a volumetric flask. Add 2 drops chloroform (CHCl_3) to suppress biological activity and store solution in borosilicate glass, preferably in the dark.

11.2.8 SULFATE SECONDARY STANDARD.

1 mL contains 100 μg of S (0.1 ppm).

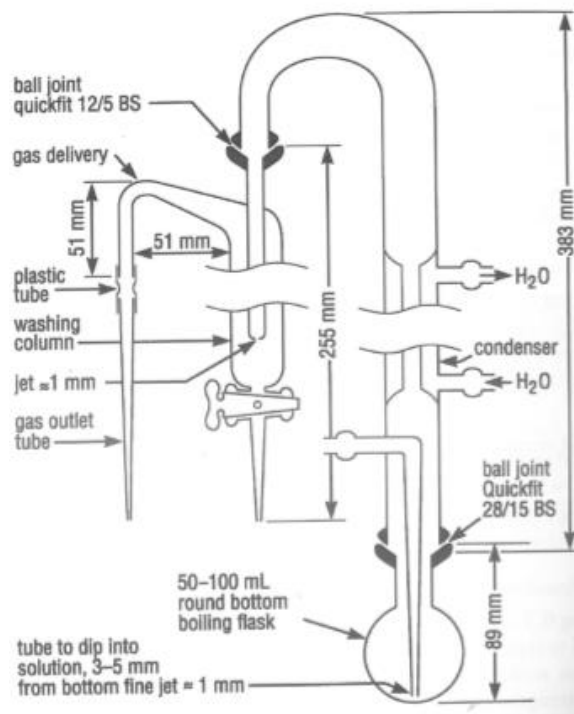
Take 50 mL $\text{SO}_4\text{-S}$ Primary Standard and dilute to 500 mL with 0.01 M $\text{Ca}(\text{H}_3\text{PO}_4)_2$ at pH 4.0 Extracting Solution. Retain after preparing working standards and use the excess to 'condition' the Johnson and Nishita apparatus (Figure 1).

11.2.9 SULFATE WORKING STANDARDS.

Prepare by adding 0, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mL of freshly prepared $\text{SO}_4\text{-S}$ Secondary Standard to separate 500 mL volumetric flasks. When diluted to 500 mL with Extracting Solution these working standards contain 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg S/L. Equivalent soil strengths for a 1:5 soil/solution ratio are 0, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg S/kg, respectively.

11.3 APPARATUS

11.3.1 The apparatus shown in Figure 1 is based on Johnson and Nishita (1952). A gas delivery tube is connected to the U tube of the gas washing column by a short piece of plastic tubing. The delivery end of the gas delivery tube is tapered, allowing it to reach the bottom of a 100 mL volumetric flask. Heating is by micro-burner via a suitable gauze. All ground glass joints should be lubricated with S-free ground-glass joint lubricant.³



³ To remove S from ground-glass joint lubricant, mix ≈ 5 g grease with 5 mL of both HI and H_3PO_2 in a 50 mL beaker. Fill a round bottom Kjeldhal flask with cold water and place it on top of the beaker to act as a condenser. Boil the mixture with frequent stirring for about 45 mins. Pour off the acid and wash the lubricant thoroughly with deionised water.

Figure 1. Modified Johnson and Nishita apparatus for calcium phosphate-extractable S (from Rayment and Lyons 2011).

11.4 PROCEDURE

11.4.1 EXTRACTION

- 11.14.1.1 Weigh 20.0 g soil (air-dry, <2 mm) into a 250 mL plastic bottle.
- 11.4.1.2 Add 100 mL Extracting Solution [0.01 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$ at pH 4.0], use a stopper, and shake end-over-end for 17 h at 25°C.
- 11.4.1.3 Centrifuge at about 3000 rpm for 20 min or filter extracts through Whatman No. 42 paper, discarding the first portion.

11.4.2 MEASUREMENT

- 11.4.2.1 Clean the Johnson and Nishita apparatus thoroughly with deionised water (detergent washing recommended following periods of storage) and drain.
- 11.4.2.2 Grease all Quickfit male joints of the apparatus with a minimum of S-free lubricant.
- 11.4.2.3 Add 10 mL Gas Washing Solution to each column and assemble the apparatus as shown in Figure 1 using Quickfit springs where necessary.
- 11.4.2.4 Turn on condenser, water and gas burners and adjust N_2 flow rate to ≈ 150 mL/min (3 to 4 bubbles/s). Check periodically.
- 11.4.2.5 To ensure H_2S saturation of the liquid system, condition the apparatus each day by adding initially 0.5 mL $\text{SO}_4\text{-S}$ Secondary Standard to the round-bottom reduction flask.
- 11.4.2.6 Add 2 glass beads and 4 mL Reducing Agent and quickly attach the flask to the condenser and heat to boiling point within 60 s.
- 11.4.2.7 Reduce the heat and allow to reflux for ≥ 30 min. (A receiving vessel is not required at this stage.)
- 11.4.2.8 Prepare a series of 100 mL volumetric flasks containing 10 mL of Absorbing Solution and 70 mL of deionised water. When the apparatus has been conditioned, raise one of these volumetric flasks under the gas delivery tube of each apparatus until the outlet almost touches the bottom of the volumetric flask.
- 11.4.2.9 Pipette a suitable aliquot of soil extract (or working standard), commonly 5.0 mL, to a reducing flask. Ensure the flask top is slightly greased, add glass beads and, immediately prior to connecting the apparatus for refluxing, add 4.0 mL Reducing Agent. Quickly connect the flask to the condenser with a Quickfit spring.

- 11.4.2.10 Heat to boiling within 60 s, then reduce heat and allow to reflux for 60 min at a low boil. Check the N₂ gas flow rate periodically during this period. When S concentrations are low, take a larger aliquot and evaporate to dryness before adding the reducing agent.⁴
- 11.4.2.11 Detach the glass delivery tube from the plastic sleeve so that the delivery tube drops gently into the 100 mL flask.
- 11.4.2.12 Pipette 10 mL aminodimethylaniline solution into a 100 mL flask, stopper and shake, then add 2 mL Ferric Iron Solution, restopper and shake.
- 11.4.2.13 Remove the glass delivery tube from the 100 mL flask with a pair of tweezers and rinse clean with deionised water. Make volume to 100 mL with deionised water and shake well.
- 11.4.2.14 Allow at least 10 min for full colour development: colour remains stable for up to 24 h.
- 11.4.2.15 Detach the reducing flask, allow it to cool, then pour the used reducing agent via a grooved funnel (to catch glass beads) into a suitable glass storage bottle for later regeneration.
- 11.4.2.16 When fully drained, reattach the glass delivery tube to the apparatus and repeat the operations on equal volumes of the extracting solution, soil extracts, and working standards.
- 11.4.2.17 When operations for the day are complete, shut down the apparatus.⁵
- 11.4.2.18 Read the absorbance of both standards and soil extract solutions in the range 660–670 nm on a suitable spectrophotometer.
- 11.4.2.19 Plot absorbance of working standards against S concentrations (or use a regression equation) to obtain S concentration of soil extracts.
- For a 1:5 soil/solution ratio and equal aliquots of soil extracts and Standard Solutions, S concentrations can be obtained directly.
- 11.4.2.20 Adjust for any reagent blank.
- 11.4.2.21 Report phosphate-extractable S (mg S/kg) on an air-dry basis.

Notes: When turning off the apparatus, concurrently turn off the gas at the supply outlet and remove the N₂ delivery tube. Next turn off the N₂ gas supply. Finally, shut off the water supply to the condenser.

⁴ Where large aliquots of soil extracts are necessary (due to low S concentration) the extract solution should be evaporated to dryness prior to the addition of Reducing Agent. Evaporation may be accelerated in an oven at 130 °C or by blowing a stream of air, washed in 4 M KOH (224.4 g/L) into the upper part of the flask.

⁵ Leaving the reducing flask connected to the condenser following the last reflux on the previous day stops impurities entering the apparatus and maintains an inert atmosphere (N₂), obviating the need to thoroughly clean the apparatus prior to use.

This manual Johnson and Nishita analytical finish can be readily adapted to measure S in clarified extracts.

11.5 CALCULATION

Prepare a standard curve of mg/L S (working standards) against absorbance.

Read off unknown as mg/L S.

$$\text{SO}_4\text{-S (mg/kg)} = (a - b) \times V/W \times \text{MF}$$

where: a = SO₄-S in sample solution (mg/L)

b = SO₄-S in blank solution (mg/L)

V = volume of 0.01 M Ca(H₂PO₄)₂ eluant (mL)

W = weight of soil

MF = moisture factor.

The simplified equation below is used for calculation of results:

For a 1:5 soil:eluant ratio (20 g soil:100 mL 0.01 M Ca(H₂PO₄)₂)

$$\text{SO}_4\text{-S (mg/kg)} = (a - b) \times \text{MF}$$

Report results to the nearest whole number.

Interpretation of results

Follow Table 14 below for ratings and interpretation of data. Critical S values of Pacific Island soils have not yet been determined, however the following are some broad ratings of phosphate-extractable sulphate measurements (Blakemore et al. 1987).

Table 14. Ratings for phosphate-extractable sulphate (from Blakemore et al. 1987).

Rating	Phosphate extractable sulphate (mg/kg)
Very high	>150
High	50–150

Medium	15–50
Low	5–15
Very low	<5

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