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Final report

Small research and development activity

project

Scoping study to assess needs and options to redevelop NAFRI's analytical laboratory

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1 Acknowledgments

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2 Executive summary

Three major bottlenecks were identified that prevented the efficient flow of samples through the NAFRI laboratory, viz.

- Lack of the type of **instrumentation** required to fully operate a soil analytical facility and the very poor state of repair of the equipment actually present,
- Insufficient training of **personnel** in soil analytical techniques and instrument maintenance, and
- An inadequate system of **sample preparation** (drying, sieving, and passage through the laboratory).

Increased efficiency would be gained by converting as many of the laboratory protocols as possible to a **centrifuge-based mode of operation**. By conducting extractions in plastic tubes, samples are compactly handled in racks, shaking of samples is simplified, separation of soil and solution is rapid and easy, as is post-analysis clean-up.

In the short term, therefore, analytical capacity has been expanded and throughput efficiency increased by the purchase and commissioning of:

- Bench-top centrifuge (28 x 50ml tubes)
- Atomic absorption spectrometer
- UV/VIS spectrophotometer with 6-cell holder

along with ancillary items viz. Vortex Mixer, pH Sensor, specialty chemicals, and custom-designed racks for centrifuge tubes and cuvettes.

It is highly desirable that laboratory personnel acquire more expertise in the understanding of soil chemical processes and why analyses are performed in a certain way, and also be able to judge whether currently selected protocols are the most suitable to provide solutions to the problems at hand. This expertise could be gained by the attendance of one of the Soil Scientists on staff at a Soil Chemistry course conducted at least M.Sc. level.

For now, however, a Soil Chemist (Gavin Gillman) attended the commissioning of the above-mentioned equipment items and then spent a week advising NAFRI laboratory staff on their operation, following protocols provided by Gillman. At the completion of this phase, key laboratory personnel attained proficiency in the operation of the atomic absorption spectrometer and the UV/Vis spectrophotometer, while all staff members were instructed in the operation and use of the bench-top centrifuge. All staff fully appreciated the efficiencies flowing from the conversion of as many analytical methods as possible to practices employing centrifuge tubes in racks. Thus, new protocols for pH, EC, Exchangeable Ca, Mg, K, Na, Exchangeable Acidity, Extractable P, Total K in Compost, and Oxidisable Carbon were conducted, each on 2 occasions, during the training period.

The third bottleneck relating to sample preparation would be removed by a reorganization of the Drying/Preparation shed, its thorough cleaning, and by the employment of a junior (High School graduate) staff member who would be trained in all aspects of soil sample preparation and who would be responsible for the maintenance of this area.

The goal of the NAFRI analytical laboratory should be to produce useful data of high quality, and this can be achieved by establishing a professional culture within its staff. This will require appropriate training in laboratory management/operation, and by a complete upgrade of the working environment including buildings, utilities, furnishings, and equipment. **An important additional benefit that would result from this upgrade would be the ability to analyse water samples**, where contamination must be kept to a minimum.

Therefore, in the medium term, it will be necessary to totally refurbish the main laboratory area by re-surfacing laboratory benches, substituting wooden furnishings and fittings with termite-resistant materials, replacing plumbing and electrical supply, refitting the balance and instrument rooms as purpose-oriented facilities. The floor level should be raised while re-tiling to provide a more comfortable bench height. Similar improvements also apply to the service wing, along with replacement of fume cupboards. Consideration should be given to the construction of an office building adjacent to the main laboratory to accommodate senior laboratory management and analytical staff.

This report discusses a rationale for providing soil and plant analytical services to end-users, and suggests a range of analyses and additional equipment, as well as personnel attributes that would be required to effect a more ambitious development in the medium term.

As part of medium-term planning, consideration could be given to the concept of developing a **country-wide network of analytical facilities comprising a National Laboratory and a number (3-4) smaller Regional Laboratories**, the latter being based on commodity or simply geographical priorities. Importantly, efficiency over a range of aspects would be gained by standardizing protocols and equipment, and the analytical laboratory at the National University of Laos (NUoL) Department of Agriculture could be linked to be a source of potential employees with experience in network instrumentation.

The above networking concept could be trialled using the NAFRI, Rice Research Centre, and NUoL laboratories, and suggestions for seeking ACIAR support to develop and implement this idea are presented as a series of Recommendations in Section 6 of this Report.

3 Introduction

3.1 Background

The NAFRI soil analytical laboratory receives about 1500 samples per year for processing; of these about 95% are soil samples. The principle chemical analytical parameters are pH, total N, organic matter content, CEC, P, K and Na, while the main physical parameters determined are bulk density, particle size distribution and gravimetric soil moisture content. Whilst all the above parameters can be determined by the lab, a range of bottlenecks precludes the lab from increasing its sample throughput. In many instances there are long delays between samples reaching the lab and their analysis. In the light of recent increased demand from ACIAR projects and the likelihood of major additional work (e.g. national soil surveys) there is a need to significantly boost the lab's capacity. In particular, LWR/2008/019 and CSE/2009/004 will place a heavy additional demand on the soil analytical lab, which will have to balance these demands with commitments from other national and donor funded activities. It is foreseeable that delays in sample analysis will result, impacting on delivery timelines of project milestones in both of the above projects.

This report summarizes no-regrets actions to alleviate some of the short-term bottlenecks, so that the lab can remain functional and in some instances increase its sample throughput and the quality of the analysis, thus enabling it to deliver on key analytical demands of ACIAR projects and other projects. In the medium term, enhancing the lab's performance will require a complete refurbishment and/or redevelopment of the facilities, supported by training and capacity building. This report scopes options for ACIAR supporting this in a future project.

From a NAFRI perspective, increasing the reputation of NAFRI analytical services is seen as a key prerequisite to attract more fee-paying clients, as well as ensuring that data generated by the lab is reliable and provided in a timely and cost-effective manner.

3.2 Terms of reference

Accordingly, a Small Research Activity was funded by ACIAR to address the following Terms of Reference:

1. Address operational and equipment related bottlenecks in the NAFRI soil laboratory that can be alleviated through short-term and cost-effective measures. This includes:
   - Prioritisation of short term bottlenecks listed under section 2 that can be easily alleviated over the coming 6 months through acquisition of equipment and lab ware
   - Determination of short term capacity constraints that can be easily alleviated over the coming 6 months through targeted training and capacity building
   - Implementation of the above measures

2. Assess options for the medium term redevelopment of NAFRI's analytical capability. This entails:
   - A more detailed stock take of equipment and capabilities in other existing laboratory facilities in NAFRI and NUoL
   - Mapping future demand for analytical services internally (NAFRI) and externally (agribusiness and mining sectors)
   - Determining human resource requirements for a staged upgrading of the laboratory(s)
   - Determining major items of equipment required for a full-scale redevelopment of NAFRI's analytical capabilities

- Determining prerequisites necessary for NAFRI to achieve lab accreditation
- Assess feasibility of a range of future modalities to manage analytical facilities:
  i) new, custom built and centralised laboratory servicing all of NAFRI
  ii) upgrades to existing laboratories to form a network of complementary facilities
  iii) collaborative arrangements with other laboratories outside NAFRI (e.g. NUoL)

3. Provide recommendations to ACIAR on possible modalities and scope of future ACIAR support to implement the options reviewed under TOR 2.

At the completion of this study, the Client's Contract was extended by the following Variation:

4. Implementation of TOR 1 by:

- Tendering and acquisition of proposed equipment items
- Installation of equipment at the NAFRI analytical laboratory
- Instruction of NAFRI laboratory staff in operation and maintenance of the equipment
- Drawing up of new laboratory protocols
- Training of laboratory staff in the conduct of the new protocols

4 Identification and Alleviation of Short-Term Bottlenecks

4.1 Overview

The NAFRI soil analytical laboratory has 9 personnel comprising 7 permanent staff and 2 contracted members. Two staff members have higher degrees (MSc in Soil Science), one being the Laboratory Director, two have a BSc in Chemistry, two have a B.Sc. in Plant Science, and one a BSc in Biology. The remaining members have High Diplomas, one in Plant Science and one in Forestry. It should be noted that there are no Soil Chemists in the team.

The main laboratory consists of two spacious general purpose areas, a small weighing room, a small instrument room, and a relatively small room for staff computing and meal breaks. Soil preparation (drying and grinding) as well as soil archival storage is housed in a separate shed of generous proportions. A third building is divided into a series of small rooms for activities such as ammonia distillation, soil digestion, chemicals storage, and glassware and equipment storage.

Although the main laboratory area appears to be structurally sound, the interior will soon need to be totally refurbished, particularly bench-top surfaces, cupboards under benches, and floor tiles. When this is done, a complete electrical wiring is advised, taking particular attention to the provision of sufficient power outlets to accommodate the increasing instrumental sophistication that will eventually occur. **In the short term, however, operations should be able to continue by providing certain items that will be described later in this report.** Some longer term issues relating to building refurbishment are discussed in section 5 of this report.

4.2 Identification of Bottlenecks and their Solution

The most obvious bottlenecks to efficient sample through-put were:

- Lack of the type of **instrumentation** required to fully operate a soil analytical facility and the very poor state of repair of some of the equipment actually present,
- Insufficient training of **personnel** in soil analytical techniques and instrument maintenance, and
- An inadequate system of **sample preparation** (drying, sieving, and passage through the laboratory).

4.2.1 Instrumentation

The minimum data set that a soil analytical laboratory specializing in soil chemical and physical analyses might produce would comprise:

- Soil pH and electrolytic conductivity (EC)
- Organic carbon
- Total nitrogen and nitrate
- Extractable phosphate
- Exchangeable basic cations
- Exchangeable acidity
- Cation exchange capacity (CEC)
- Total phosphorus and potassium
- Particle size analysis (PSA)
- Moisture content

- Bulk density

Additional analyses that might be required by particular clients include:

- Free iron and aluminium
- Extractable nitrate and ammonium
- Micronutrients
- Extractable sulphate
- Moisture retention curve (at a minimum, 1kPa and 150 kPa)

To put the capability of the NAFRI soil analytical laboratory into perspective, its ability to meet this minimum data set criterion was summarized as follows:

Soil pH and EC

A very adequate pH meter (Jenway 3520) was inoperable undoubtedly owing to a faulty sensor. A small hand-held EC meter with a bulky sensor system gave satisfactory results.

A hand-held EC meter was operating satisfactorily.

Organic Carbon

Walkley and Black carbon determination was satisfactory though a colorimetric finish would be desirable.

Total nitrogen

There are two block digesters with one away for repair and the other failing during my visit, and so down for repair. The steam distillation apparatus is old but is processing samples at about 30/day.

Nitrate

ACIAR project LWR/2008/0919 supplied a MERCK Reflectometer which is being used for nitrate (and potentially ammonium) analysis. The technique is relatively simple, but needs further comparative testing with conventional testing, which is underway in LWR/2008/0919. Test strips and some of the ammonium reagents need to be sourced from outside Laos.

Extractable phosphate

The Bray 2-P method permitted very short extraction time but filtering of the suspension can be prolonged.

The spectrophotometer (Jenway 6320 D) was unstable, resulting in much time wasted in calibration and re-calibration and the production of unreliable results.

Exchangeable basic cations

Exchangeable K and Na were determined on an ancient Russian built flame photometer with uncertain results.

Exchangeable Ca and Mg can theoretically be determined by EDTA titration, but were not done.

Cation exchange capacity

This is determined by a method (Ammonium acetate) that is increasingly recognized as inappropriate for highly weathered soils. Access to the ammonium still is difficult when total nitrogen analysis is in progress.

Exchange acidity

The soil was extracted in coke bottles by shaking them on a unique-looking end/end shaker in batches of 8. The suspensions were then filtered before an aliquot is taken for titration with alkali. This is a cumbersome procedure for only 8 samples.

Particle size analysis

The hydrometer method, (and sometimes the pipette method) is used and appears to be carried out quite efficiently. We observed a batch of 38 samples being processed.

Gravimetric soil moisture and bulk density

Standard procedures are used and reliable data is being generated. However, at times the balance and oven space for drying might be bottlenecks.

In summary, pH, EC, Walkley and Black carbon, PSA, gravimetric soil moisture and bulk density determinations were conducted reliably with the available equipment. However the other methods of analysis were either slow, unreliable, or not able to be carried out.

To alleviate this instrumentation bottleneck, the items listed below, within budgetary limits, were recommended after considering the following issues:

(a) Suitability of analytical methods

Methods used in soil analysis should reflect our best understanding of soil properties and processes in order to provide the best advice on soil management.

(b) Operational efficiency

By choosing appropriate equipment, analyses can be streamlined when targeted species are extracted efficiently and then determined by the most appropriate instrumental technology, be it colorimetry, flame spectroscopy, titration etc. This consideration of efficiency should also take into account issues such as the separation of soil from the extracting solution (when required) and the cleaning of laboratory vessels at the completion of the determination.

It was therefore recommended that the NAFRI laboratory convert to a centrifuge-based system for as many analytical procedures that could be appropriately accommodated in this way. The centrifuge tube (polypropylene) becomes the preferred vessel for the major operations of extraction, separation of soil from solution, as well as for any dilution steps required prior to actual analysis.

The analyses that can be adapted to a centrifuge-based mode include:

- pH and EC
- Extractable phosphate
- Exchangeable basic cations
- Exchangeable acidity
- Cation exchange capacity
- Extractable nitrate and ammonium

These methods, as well as those pertaining to the determining of organic carbon and total nitrogen rely heavily on a colorimetric or flame spectroscopic finish, and such instrumentation was either lacking or severely compromised in the NAFRI laboratory. It was therefore proposed that the following items have the highest priority in the equipment ordering list: (see Appendix 1 for specifications):

- Bench-top centrifuge with capacity for 16 x 50ml centrifuge tubes.
- Atomic absorption spectrophotometer with lamps for the determination of Ca, Mg, K, and Na. (also for Cu, Zn, Mn and Fe or Bo if funds are available)
- UV/VIS Spectrophotometer with sipper and flow cell accessories.

Other urgently-needed items that could remain within or close to the budget limit included:

- Vortex mixer
- Semi-micro Markham still
- pH sensor for Jenway pH meter
- 2 x top-pan balances reading to 2 decimal places
- Spare distilled water generator
- Large diameter 2mm stainless steel sieve

4.2.2 Personnel Training

In observing staff performing their daily duties in the NAFRI laboratory one detects an air of quiet determination to produce analytical results under trying circumstances. Equipment deficiencies have already been mentioned, but in some areas there is an absence of understanding of the rationale behind some of the laboratory protocols. This is not universally true, with total N and PSA for instance being conducted efficiently with the available equipment. Problems can be attributed to the fact that the laboratory does not have a Soil Chemist.

The situation could be resolved by having at least one of the two Soil Scientists attend a Soil Chemistry program at an appropriate university. An alternative could be the appointment of a Soil Chemist, but the present complement of 9 personnel is adequate to handle the number of samples projected in the short to medium term, provided that the efficiencies recommended in this report are implemented. Attention must also be applied to appropriate training of staff in the maintenance of equipment and the more general notion of the introduction of a **Maintenance Culture.** The latter issue should be a priority for the laboratory Director, who should also receive specialist training in Laboratory Management.

As it is probably not feasible to send staff out of country for training in laboratory practices and procedures, a recommendation for the short-term would be to engage the services of an experienced soil chemist to work with personnel in the NAFRI laboratory for a sufficiently long cumulative period, perhaps 4-6 weeks. An initial period of 2 weeks coinciding with the installation of the three major instruments ensured that staff was fully acquainted with its operation and maintenance.

4.2.3 Sample Preparation and Handling

The building that has been set aside for drying and grinding of soil samples is quite suitable for the purpose. This spacious shed is divided into 3 main sections:

- A large area for soil drying
- A slightly smaller area for archiving of soil samples
- A relatively small area for soil and plant grinding and sieving

The soil drying area contains racks that are very suitable for air-drying soils, but samples are stacked in a chaotic manner with the soils drying in opened plastic bags used for soil field sampling. Although the shelves have sufficient vacant space, a large number of samples are simply being laid out on the floor of the building. Also, there are a large number of soil survey archival samples in this soil drying section that could easily be re-located to the soil archival storage section.

There is a large assemblage of unidentified objects (belonging to other organisations on the NAFRI campus) in the soil drying area that contributes to a general state of untidiness. If these were to be removed, or at least the useful items stacked properly, and if the

archival soil samples were re-located, the area could be thoroughly cleaned and then maintained.

Many soils have obviously arrived in a very moist condition and have dried to form a bolus with concrete-like consistency. This creates unnecessary work and lost time in breaking up these samples prior to sieving. Also, the 2mm sieve used is only about 200mm in diameter restricting the speed of sieving.

It would be desirable to establish a drying system where incoming samples are transferred to inexpensive plastic trays (e.g.30 cm x 20 cm) with each sample accompanied by a paper ticket containing identifying information. Samples arriving in wet condition should be broken up as much as possible, and as soon as they dry to a friable condition they should be broken down by hand. If the paper ticket is in intimate contact with the soil, it can be used as an indicator as to when the soil is air-dry. Following such a protocol will allow soils to be sieved in a rapid manner.

After passing through the 2mm sieve, and a sub-set through a 0.5mm sieve, the samples can be stored in zip-lock plastic bags on plastic or wooden trays for transportation to the laboratory. The present system of storing soil samples in large trays in the balance room should be discontinued and this room should house only analytical and top-pan balances.

4.3 Equipment Procurement and Costs.

It was believed desirable that this initial purchase of equipment items within a very limited budget be placed with a single supplier with the capacity to provide installation, initial training, and on-going servicing, and to be able to ship the items in a single consignment. A tendering process established that GBC Scientific Equipment (Qld) would meet these requirements, that the capability of the 3 priority items this company could provide (see below) would quite adequately perform the desired functions, and that they would be acquired within budget.

Priority Items	***AUD***
1. Benchtop Centrifuge. Firlabo SW12 with rotor, cups and adaptors to take 16 x 50ml Falcon skirted tubes.	7,200
2. Atomic Absorption Spectrophotometer. GBC XplorAA with extended PMT range for K, and Hollow Cathode Lamps for Ca & Mg and Na & K.	15,100
3. UV\VIS Spectrophotometer. Metertech SP8001 with 6-place cell	8,200
4. Desk-top computer to run AAS and collect data from AAS and Spectro.	1,100
5. Centrifuge Tubes and Racks	320
6. Essential chemicals	1,000
7. GBC Principal to visit Vientiane for 2 days installation and training	5,050
8. Freight on items by air Brisbane-Vientiane	2,200
Smaller Items in order of priority	
9. Vortex Mixer Genius 1ka	580
10. pH Sensor for Jenway pH Meter	250
TOTAL	**41,000**

A number of small items, totalling about $5,000, that would significantly improve operations in the short term had to be excluded for budgetary reasons.

4.4 Other Operational Issues

4.4.1 Laboratory Tidiness and Unused Equipment

There are a number of equipment items in the main analytical laboratory that are unserviceable or simply un-used. The problem is compounded by having an equipment storeroom that is choked with items that will never be used. There appears to be a reluctance to remove from the campus anything that is no longer useful, but it is imperative that this be done to allow the equipment store to be properly stocked with back-up instruments or spare parts. The same situation applies to the glassware store, where useful items should be stored and others discarded. Large, potentially useful items such as the two sterile air cabinets standing in the main laboratory could be covered and placed in the soil drying area once space there has been rationalized and the area cleaned.

4.4.2 Chemical Storerooms

The two chemical storerooms, one for liquids and the other for dry chemicals **present a highly dangerous situation that if not addressed in the very near future will almost certainly result in personal injury**. In the liquid store, labels have disappeared from what appears to be highly corrosive acids such as nitric and perchloric acid, while in the dry chemical store, tins are rusting and many items are un-labelled. It is not clear whether there exists in-country an organization with the capacity or expertise to dispose of these un-labelled, hazardous materials.

At the same time however, there are items with high replacement value that could be salvaged and re-labelled if necessary, and relocated in-store once unwanted items have been removed and the room thoroughly cleaned.

4.4.3 Soil Digestion Room

One of the two fume cupboards in the room is still functioning. Although there is an extractor fan in an end wall to remove any residual fumes, it appears that vented fumes re-enter the room through broken louvers. This problem should be addressed by replacing the missing glass louvers and extending the fume pipes.

4.4.4 Allocation of Space in the Main Laboratory

It is suggested that the dirtier and mechanically-oriented aspects of soil analysis be conducted in the rear large laboratory. The type of activities that could be conducted here include particle size analysis, oven drying, muffle oven heating, end/end shaking, centrifugation etc. The front large laboratory could be reserved for small-scale instrumental analysis such as pH and EC measurement, semi-micro still, sample dilutions, standards preparation, and so on. The balance room should house all of the balances and soil samples awaiting analysis should not be stored in this room. The room currently housing the flame photometer could adequately accommodate the AAS and possibly the spectrophotometer both connected to a computer.

4.5 Equipment Installation and New Protocols

Following an initial 10-day mission to assess short-term bottlenecks to NAFRI laboratory operations, a second 14-day mission was launched. The first week of this 2-week mission was devoted to the commissioning of the pre-delivered three major equipment items and the introduction of staff to them. By arriving a day before the instrument installer, Gillman was able to site the machines and source local fittings and accessories, allowing the

installer to fully allocate his time (3 days) to ensuring that the machines were functioning according to specification, and to instructing key staff on their use and maintenance.

In a remarkably short time-span staff were confidently operating the AAS, despite no previous experience with this technology, though practices for optimizing analytical conditions will come with experience as they begin to routinely process samples. Operation of the UV/Vis spectrophotometer was even less of the problem owing to previous use of this type of (less sophisticated) instrument.

Unfortunately, during the last day of the installer's attendance, the centrifuge failed during a routine run-up, possibly as a result of poor power supply in the lab. Despite concerted effort to diagnose the problem in consultation with the manufacturer's engineers, the fault was not identified, and the machine has been re-packed for transport to Singapore for repair under warranty. Staff members, however, have received sufficient instruction on the use and general maintenance of this uncomplicated device.

The second week was fully utilized in actually conducting (twice) newly introduced protocols (See Appendix 1. Part 7) for the analysis of soil pH and EC, Exchangeable Ca, Mg, K, and Na, Exchangeable Acidity, Available P, Total K in compost, and Walkley/Black C. In the majority of these methods samples are weighed into 50ml plastic centrifuge tubes contained in purpose-built racks where the processes of extraction, soil/liquid separation, and extract dilution can be easily performed in batches of 16 samples, followed by rapid cleaning and drying of the tubes without removal from the racks.

During periods of waiting (shaking, settling etc) the opportunity was taken to conduct mini-seminars on the theory governing ion-exchange and CEC, on the principles of atomic absorption spectroscopy, and on general laboratory operations such as pH meter calibration, dilution using auto-pipettes, and operation of the vortex mixer.

Senior staff should now be able to supervise the use of the newly-acquired equipment and the conduct of new protocols, but have been assured of on-going advice by E-mail should the need arise.

Figure 1: Installed AAS (left) and new spectrophotometer in operation (right)

5 Medium Term Development of Analytical Capacity

5.1 Equipment and Staffing Assessment for National Laboratory

The following simple model, framed as 5 questions is useful when assessing equipment and staffing requirements for a national soil and plant analytical facility:

- What do end-users need?
- What operational methods are required to gather relevant information?
- What equipment is required to conduct these operations?
- What are the personnel requirements?
- How will results be interpreted and communicated to clients?

This model constitutes a 'logical loop' by beginning and ending with end-user needs while along the way identifying the material and human resources that NAFRI would require to address those end-user needs.

5.1.1 End User Needs

The NAFRI analytical laboratory currently receives samples from a range of clients that include Government Departments, International projects, plantation operations, and local farmers. Around 1500 samples per annum are processed but it is believed that if operations could be streamlined and reliable results from better instrumentation produced, potential customers would gain greater confidence in using the service. In the short to medium term, sample numbers could increase to 2000-3000 per annum.

One potentially large source of samples would be National Soil Assessment studies if approved by Government, but the increasing activity in rubber, tree planting, coffee etc could also lead to increased private sector sample requests more directly related to plant nutrition. Analyses to support fertilizer accreditation will also increase. There will also be requests for plant analysis as well as for the analysis of fertilizer products given the increasing popularity of organic fertilizers.

The information that will usually be necessary to satisfy the needs of this diverse client range will include:

(a) for soils

Particle size analysis (soil texture), soil pH, electrolytic conductivity (EC), cation exchange capacity (CEC), availability of macro- and micro-nutrients, soil carbon and nitrogen status, content of heavy metals.

(b) for plants

Total macro- and micro-nutrients and toxic metals

(c) for fertilizers

Total nitrogen, phosphorus, and potassium, heavy metal content, total and oxidisable carbon, nitrate and ammonium nitrogen, and micronutrients.

5.1.2 Methods

The range of analytical methods that could usefully be employed would comprise:

- Particle size distribution (soil texture) by the pipette method (requiring new pipette)
- Soil pH, electrolytic conductivity, exchangeable basic cations, and cation exchange capacity by the method of compulsive exchange

- Titratable acidity by KCl extraction
- Organic carbon in soils, plants, and organic fertilizer by the method of Walkley and Black.
- Nitrogen in soils and plants, and total N, nitrate and ammonium in fertilizer by Kjeldahl method
- Total phosphorus in soils and plants by acid digestion, and extractable phosphorus in soils and fertilizer precursors by Olsen or Bray methods.
- Extractable Si in soils, and total Si in fertilizer by acid digestion.
- Total potassium in plants and fertilizer by nitric/perchloric acid digestion.
- Micronutrients (Fe, Mn, Cu, Zn) in soils by DTPA extraction, and in plants by acid digestion.
- Micronutrients (Fe, Mn, Cu, Zn, Al, B) in fertilizer by acid digestion
- Heavy metals (e.g. in sludge) – Cd, Pb, Cr, Ni, Sn, Hg, As – by acid digestion

5.1.3 Equipment

With the list of methods agreed, the major and minor items of equipment needed to conduct the above analyses can now be identified:

NOTE: * = Already accounted for in previous analytical list

Particle Size Analysis

1 x Analytical Balance (4 place)
1 x Drying Oven
20 x Settling Cylinders (1 litre)
50 x ceramic dishes (50 ml)
1 x 50 micron Sieve (100mm diam.)
Pipette system for taking soil solution at different settling levels

pH, EC, Cations, CEC

1 x Atomic Absorption Spectrophotometer (Flame) *
1 x Low Speed Centrifuge (16 x 50ml capacity) *
1 x Shaker
1 x Top Pan Balance (2 decimal places)
1 x pH Meter *
1 x EC Meter
1 x Vortex Stirrer *
30 x Centrifuge Racks *
1000 x falcon skirted centrifuge tubes *

Titratable Acidity

1 x Digital Burette
2 x Magnetic Stirrers
Centrifuge *
Centrifuge racks and tubes *

Carbon

1 x Uv/vis Spectrophotometer
1 X Block Digester

30 x Erlenmeyer Flasks

Nitrogen

2 x Kjeldahl Distillation Apparatus
2 Block digesters with 40 places *
Digestion Tubes *
Digital Burette *
Spectrophotometer *
pH Meter *
2 x Fume cupboard

Total Phosphorus

1 x Dilutor
Block Digester *
Spectrophotometer *

Available Phosphorus

Centrifuge *
Spectrophotometer *
Shaker *
Centrifuge Racks *

Silicate

1 x Muffle Furnace
2 x Stirrer Hotplates
Spectrophotometer *

Sulfur

Spectrophotometer *
Shaker *
Centrifuge *
Centrifuge Racks *

Micronutrients

Atomic Absorption Spectrophotometer *
Shaker *
Centrifuge and centrifuge racks and tubes *

Soil and Plant Preparation

1 x Soil Grinder
1 x Plant Grinder
1 x Stainless Steel Sieve 2 mm (40 cm diam.)

To this summary of equipment specifically needed for the listed analyses there needs to be added a list of supporting items (eg. water stills, ovens, grinders. etc) that are not specific for any method but form part of the basic 'infrastructure' of a modern analytical

facility. This leads to the following summary of the equipment needs of an upgraded NAFRI soil and plant analytical facility:

Analytical Balance (4 decimal places)	1
Atomic Absorption Spectrophotometer	1
Autopipetter	4
Block Digester (20 place)	2
Bottletop Dispenser	9
Centrifuge Low Speed 16 x 50ml capacity	1
Centrifuge Tube Racks	20
Centrifuge Tubes 50ml Falcon skirted	500
Ceramic Dishes (50ml)	20
Desiccator	3
Digestion Tubes	400
Digital Burette	1
Diluter	1
Drying Oven (large)	1
Drying Oven (Small)	1
EC Meter	1
Kjeldahl Distillation Apparatus	2
Laboratory Stirrer	1
Muffle Furnace	1
pH Meter	2
Plant Grinder	1
Refrigerator	1
Settling Cylinders (1 litre)	40
Shaker	1
Sieve 50 micron (100mm diam.)	1
Sieve Stainless Steel 2mm (30cm diam)	1
Soil Grinder	1
Spectrophotometer (Uv/Vis)	1
Stirrer Hotplate	3
Top Pan Balance (2 decimal places)	2
Vacuum Pump	1
Volumetric Flasks (100ml)	100
Vortex Stirrer	1
Water Bath	1
Water Demineraliser	1
Water Still 4 l/hr	2

5.1.4 Personnel Requirements

It is difficult to predict future staff needs as this will depend on the successful implementation of improved methods along with an enhanced reputation for producing reliable results that will lead to end-user trust and submission of increasing numbers of samples. In the meantime, however, the opportunity should be taken to improve the soil chemical knowledge of existing staff as well as the provision of scholarships for students that would allow them to seek higher degrees in Soil Chemistry at appropriate universities.

It is recommended that two key personnel form the nucleus of a National Soil Analytical facility – a Leader trained to PhD level in Soil Chemistry and a Deputy Leader trained to MSc level in Soil Chemistry. These Key Personnel could then be involved in the in-house training of junior staff. It would also be desirable that one of the Key Personnel receive training in Chemistry Laboratory Management.

As will be discussed below, enhancement of the interpretative value of results would be obtained by establishing a Database that combines analytical results with client outcomes. **It is recommended that a Database Manager be appointed** to set up a system of streamlining the capture of daily laboratory data as well a process for obtaining client feedback on outcomes based on the interpreted results, combining all information into a Master Database.

Operations would be greatly facilitated if it were possible to engage a Laboratory assistant (High School graduate) to be trained in all aspects of soil sample preparation, and be responsible for orderliness in the soil storage/drying/preparation shed. Should there be periods of low activity in respect of sample arrivals, this person could act as a cleaner in the main laboratory or even be engaged in simple laboratory routines

5.1.5 Communication with End-Users

Apart from the production of high quality results that should be expected following the proposed upgrading of equipment, analytical protocols, and general laboratory practices, to gain the trust of potential users of NAFRI services it will be necessary to provide an interpretation of the results within the limits of current knowledge.

The keys to providing an interpretation service are an efficient system for collecting and collating data from the laboratory, and combining this with feed-back from the client into a Database. There therefore has to be a 2-way interaction between the service and the customer.

The process begins with a discussion between the end-user and the laboratory representative when samples are submitted to gain information on why the customer requires a particular analysis. It continues with the client receiving a certified copy of results (that have been added to the Database) with interpretation and recommendations. There should then be a procedure for obtaining feed-back from the client as to whether the advice was followed and with what outcome. This feed-back information would then be added to the Database in order to constantly improve the interpretation and recommendation phase of the process.

5.2 Other Soil Analytical Facilities in Laos

It appears that the only other soil/plant analytical laboratories in the country are located at NAFRI's National Rice Research Centre at Napok and the NUoL Department of Agriculture at Nambung. There is a Livestock Research Centre laboratory that examines feedstock, seed quality etc. but does not analyse soil and fertilizer.

5.2.1 Rice Research Centre Analytical Laboratory

This smallish building is surprisingly well equipped with a range of analytical instruments that are of recent acquisition:

- Fume cupboards (2) but only one operational
- Block Digester (40 place) built in Thailand in working order
- UV/VIS Spectrophotometer (Shimadzu 160 IPC) with sipper, in working order but attached computer for data collection has software problems
- Flame photometer (Sherwood 410) in working order

- Automatic ammonia still (Gerhardt Vapodest) not operational but problem might be minor as it failed while still under warranty.
- Analytical balance and top-pan balance in working order
- pH meter (Schott) not operational but appears to only be a faulty sensor
- EC meter (Schott) probably operational but incorrect power plug fitted
- Convection oven (Precision Gravity) in good condition but incorrect power plug fitted
- Water still (Corning MP-45 megapure) operational but delivering only 1 litre per day.
- Drying oven operational but faulty door latch

The laboratory has as its only staff member a graduate from NUoL with a BSc that included some laboratory training. As will be seen below, and laboratory training would have been basic and would account for the progressive deterioration in analytical capacity that is obviously occurring at the Rice Research Centre laboratory

5.2.2 NUoL Department of Agriculture Soil Analytical Laboratory

This laboratory, staffed by a Manager and an assistant, is urgently in need of an equipment upgrade if it is to play any role in student education. So much of the equipment is unserviceable that only functioning equipment is listed below:

- Spectrophotometer (Spectronic 20D)
- pH meter (Schott Lab 80)
- Macro Kjeldahl 6-place digestion rack with 3 places operating
- Heating Mantle rack 4-place
- Top pan balance (Ohaus Precision Plus)
- Ammonia still (small)
- Water Still (Merit W4000) delivering 5 litres per day.

Water supply to the laboratory is intermittent adding to difficulties in operating the stills and cleaning.

There is a general lack of glassware, but useful items for student use could be sourced from the over-abundant store of glassware at the NAFRI facility if the latter store is rationalized.

The Manager believes that Atomic Absorption Spectroscopy is a high priority and also a UV/VIS Spectrophotometer to replace the inadequate Spectronic machine that is still functioning. There is also a need to introduce plant analysis, and these two instruments would facilitate that initiative.

5.2.3 Relationships between Centres

Currently, there seems to be little exchange happening between the three laboratories. As the demand for soil and plant analytical services increases in Laos, it would be logical to have co-ordination between these three institutions, and linkage in the first instance could be in the form of:

(a) Standardization of Laboratory Methods

Standardization of methods allows laboratories to engage in sample exchange for quality control as well as allowing inter-institutional discussion of analytical problems, coordination of staff training programs, and consistent interpretation of advice to clients

(b) Standardization of Laboratory Equipment

Repair and maintenance in Laos will be a serious concern for laboratory managers well into the future owing to the difficulty of acquiring spare parts and the unavailability of

technicians. If instrumentation is standardized, a single technician in the 'network' could be trained to be responsible for a range of instruments, with a store of essential spare parts assembled and maintained at the National Laboratory.

(c) Capacity Building.

If there was a linkage between NAFRI and Rice Research Centre, perhaps as National Laboratory and Regional Laboratory, with coordinating and mentoring processes established, this could provide a template for the eventual introduction of other Regional Laboratories that would be established to service particular agricultural sectors or simply other geographic regions.

The primary source of personnel for what could become a network of analytical facilities throughout the country would be students graduating from NUoL. Therefore, the soil analytical laboratory at the Department of Agriculture should logically be included in the above-mentioned standardization activities even though the institutional linkage might not be as strong as that between NAFRI and the Regional Centres. This concept is further promoted in Section 6.

5.3 Building Upgrade at NAFRI

In the authors' opinion, the existing structures could well service NAFRI's requirements as a National Soil and Plant Analytical Facility into the future. There is, however, an urgent need to totally refurbish almost all of the internal space, and to consider the provision of appropriate office space for laboratory staff.

5.3.1 Main Analytical Laboratory

- Resurface laboratory benches with softer material than tiles (e.g. PVC sheeting)
- Re-plumb water supply
- Upgrade upper shelving on island benches and install multiple power outlets on them. Likewise install multiple power outlets on walls near peripheral benches.
- Replace wooden fixtures such as cupboard doors and shelves with termite resistant material such as aluminium
- Raise floor level to provide a more comfortable bench height.
- Reorganize balance room to accommodate only balances on a solid bench of a height that allows analyst to be seated while using the analytical balance.
- Refurbish the Instrument Room and add sufficient power outlets for expected range of equipment
- Provide desk spaces for analysts

5.3.2 Distillation/Digestion/Storage Wing

(It is assumed that storage areas have been addressed as discussed under Section 4)

- Replace fume-hoods in the distillation and digestion rooms
- Resurface benches in the distillation room and provide desk space for analysts
- Replace wooden fittings (cupboards, shelves etc) with termite resistant material

5.3.3 Soil Drying/Preparation/Storage Shed

(It is assumed that the improvements discussed under Section 4 would have been completed)

- Install a mechanical apparatus for grinding and sieving soil
- Fit out the area currently occupied by IRD as an office for the storage of sieved soils

5.3.4 Office Space for Laboratory Personnel

It is recommended that a small office be constructed adjacent to the main laboratory to accommodate laboratory managers (key personnel mentioned above) as well as senior analysts. This would then allow the current space to be used exclusively for staff meal breaks.

5.4 Accreditation

NAFRI requested that the scoping study also consider the feasibility of the NAFRI laboratory acquiring accreditation. It is important to understand that the term 'accredited laboratory' is not accurate, as the real purpose is that a laboratory be accredited for a specific list of methods. These methods may be of national, regional, or international standard devised by relevant technical bodies, but there is also scope to develop new methods or modify existing ones, and to provide reasoned arguments for their adoption to suit particular circumstances.

An accreditation application can only commence after a laboratory is organized to demonstrate that it can conduct its protocols in an efficient and reliable manner, and detailed documentation is also required. In light of the extensive building refurbishment and equipment acquisition needed to achieve this efficiency, the initiation of the accreditation process should only be undertaken in the medium term.

The legal implications of some analytical results, such as the certification of commercial fertilizer formulations, support the advisability of using accredited methods in this instance. At the same time, however, there is a range of soil analyses where the level of interpretation for management purposes is so broad that the necessity for seeking methods accreditation is less pressing. An alternative approach, in this case, is to have a system of internal reference sample testing, as well as inter-laboratory sample exchange programs, that ensure that on a continuing basis, the laboratory is confident that soil analytical results are reliable and within interpretative norms. This is a far less expensive approach than that involving accreditation.

6 Actions and Recommendations

In general terms, soil and plant analytical capacity in Laos would be strengthened and expanded by:

- Providing support for the medium-term redevelopment of the NAFRI laboratories as indicated in the section 5 of this report
- Assisting NAFRI to develop the concept of a network of analytical facilities throughout the country in a trial involving NAFRI, Rice Research Centre, and NUoL soil analytical laboratories.

Specific recommendations and actions have been set out in three sections:

- Actions to alleviate short term bottlenecks utilising the 40,000 AUD funding provided by ACIAR
- Recommendations to help NAFRI redevelop its analytical facility in the medium term
- Recommendations to ACIAR on the scope of a possible follow-on project to support the medium term redevelopment of NAFIR's analytical facility

6.1 Actions and Recommendations to Alleviate Short Term Bottlenecks

Action 1

The process of converting operations in the NAFRI laboratory to a centrifuge-based sample handling system to enhance sample throughput in a cost-efficient manner has begun.

Action 2

The following items have been purchased:

- Bench-top centrifuge with capacity for 28 x 50ml centrifuge tubes.
- Atomic absorption spectrophotometer with lamps for the determination of Ca, Mg, K, and Na.
- UV/VIS Spectrophotometer with 6-place cell holder.

Other items that were within or close to the budget limit were also procured:

- Vortex mixer
- pH sensor for Jenway pH meter
- Essential chemicals
- Centrifuge tubes and racks
- Desk top computer and printer

Action 3

The major equipment items within a very limited budget were placed with a single supplier with the capacity to provide installation, initial training, and on-going servicing, and ability to ship the items in a single consignment.

Action 4

A variation of the scoping study provided additional funds to engage the services of an experienced soil chemist to work with personnel in the NAFRI laboratory for an initial period of 2 weeks coinciding with the installation of the three major instruments. This ensured that staff would be fully acquainted with their operation and maintenance.

Recommendation 1

A general clean-up of the soil sample storage area and laboratory work areas should be undertaken as matter of urgency undertaken to maximise and reallocate space within the laboratory as suggested in section 5. Disposal of old and potentially hazardous chemicals should be treated with a very high priority.

Recommendation 2

Attention must also be applied to appropriate training of staff in the maintenance of equipment and the more general notion of the introduction of a **Maintenance Culture.** The latter issue should be a priority for the Laboratory Director, who should also receive specialist training in Laboratory Management.

Although significant progress was made in laboratory staff training related to the new equipment and new protocols, further training in efficient operation and results recording and collation is required. Currently, the scoping study does not envisage this in an extent required to ensure that the 40,000 AUD investment into new equipment will be maximised.

6.2 Recommendations to Help NAFRI Redevelop its Analytical Facility in the Medium Term

Prior to any further investment in medium term measures, the implementation of the recommendations listed in section 6.1 should be monitored and reviewed to ascertain their completion and effectiveness. Only then is it advisable to proceed with the following recommendations.

Recommendation 3

An architect should be engaged to work with NAFRI personnel, perhaps with the assistance of a Soil Chemist advisor, to draw up plans for the refurbishment of the NAFRI buildings, and the construction of office space for laboratory personnel.

Recommendation 4

An equipment list, based on that indicated in Section 5.3, should be agreed and tenders placed for purchase, transport, installation, and training on use of instruments.

Recommendation 5

Two staff members should be identified as Key Personnel, and that one be trained to at least MSc level in Soil Chemistry, and that one receive additional training in Laboratory Management.

Recommendation 6

A Database Manager should be appointed and if necessary receive training in computational methods of collating soil-related data.

Recommendation 7

Following the identification of major equipment items to be purchased, an Instrument Technician should be appointed, and sent for training at the various manufacturing headquarters.

Recommendation 8

Two separate lists of equipment, suited to the needs of a Regional Laboratory (Rice Research Centre) and suited to the needs of teaching institution (NUoL) but matched to

the list agreed under Recommendation 8, should be included in the above-mentioned tendering process.

Recommendation 9

Should the recommendation to establish a trial analytical network be accepted, steps should be taken to recruit an additional 2 Soil Scientists, and to begin their training in Soil Chemistry (MSc) with the aim of appointing them to the RRC and NUoL laboratories.

6.3 Scope of a Possible ACIAR Follow-on Project

It is unlikely that NAFRI will be able to find the resources necessary to carry out many of the recommendations listed in section 6.2. At the same time, many of these recommendations lend themselves to implementation through an ACIAR–supported intervention. The below considerations are designed to provide some initial guidance to ACIAR and NAFRI on possible scope, objectives and modality of such a project. We also provide very preliminary budgetary estimates for such a project.

We suggest staging the project, stage 1 focussing on the redevelopment of the main NAFRI analytical laboratory. If this stage is successful, it is proposed to move to a second stage aimed at building a national network of lab facilities across NAFRI and NUoL.

Project aim

To support NAFRI develop and increase its laboratory analytical capabilities to an internationally recognised level of quality assurance

Project objectives

1. To refurbish the NAFRI (and NUoL) analytical laboratory(ies) and equip it (them) with modern instruments
2. To train NAFRI (and NUoL) laboratory staff in modern laboratory procedures
3. To review, select and test a suite of soil and plant chemical and physical analysis methods tailored to soil and agricultural research needs in Lao PDR
4. To integrate the NAFRI analytical laboratory into an international network of laboratories to achieve international quality assurance standards

Possible Australian lead organisations or consultants

- QLD Department of Environment, and Resources Management DERM (Dr Phil Moody)
- University of Queensland (Dr Neal Menzies)
- James Cook University (Dr Paul Nelson)
- Australian consultants (Dr Gavin Gillman)

Lao PDR partners

- NAFRI
- NUoL

Project duration

- Stage 1 – redevelopment of NAFRI main analytical lab 2 years
- Stage 2 – development of regional lab network (RRC and NUoL labs) 1 year

Project budget envelope Stage 1	***AUD***
• Australian commissioned organisation (salary, operating, travel, training)	350,000
• NAFRI (salary, operating, travel, training)	150,000
• Costs for NAFRI main analytical lab refurbishment	300,000
• Costs for NAFRI lab equipment	250,000
Subtotal	**1,050,000**

Project budget envelope Stage 2	***AUD***
• Australian commissioned organisation (salary, operating, travel, training)	200,000
• NAFRI (salary, operating, travel, training)	75,000
• NUoL (salary, operating, travel, training)	75,000
• Costs for RRC lab refurbishment	100,000
• Costs for RRC lab equipment	125,000
• Costs for NUoL lab refurbishment	100,000
• Costs for NUoL lab equipment	150,000
Subtotal	**825,000**

TOTAL	**1,875,000**

NOTE: detailed cost breakdowns for the lab equipment costs were prepared as part of this report and can be obtained from Gavin Gillman.

7 Appendixes

7.1 Appendix 1: New Laboratory Protocols

Introduction

A modern Soil Analytical Laboratory should be capable of conducting analyses that provide an assessment of the general 'health' of the soil being examined. The analyses should identify the chemical and physical limitations to the growth of specific crops, and form the basis for recommendations for fertilizer and amendment practices.

This document provides information on the transformation of some existing NAFRI protocols to methods that are based on the use of a centrifuge, in order to improve efficiency. Treatment of soil samples in centrifuge tubes whenever possible leads to high efficiency in extractions, soil/liquid separation, dilution, and post-analysis cleanup. The use of large numbers of beakers, volumetric flasks, filter papers, etc, and their subsequent cleaning is avoided.

Protocols have been written for 5 of the 6 plant macro-elements (N, P, K, Ca, Mg), as well as for the harmful element Al. There is also an alternative method for end-point detection in the determination of organic carbon by the method of Walkley and Black.

Once familiarity is established in the use of three new instruments that have been provided, viz. Atomic Absorption Spectrometer, UV/Vis Spectrophotometer, and Bench-top Centrifuge, and also with the use of racks of centrifuge tubes for extraction and dilution, the range of determinations can be extended to such things as direct measurement of CEC, micronutrient extraction (Cu, Zn, Mn, Fe, etc), and total NPK in fertilizer, compost, and plant material.

Determination of pH_{H2O} , EC, and Exchangeable Ca, Mg, K, and Na

(Note: This method can be extended to include pH_{BaCl2} and CEC).

Reagents

0.2M $BaCl_2$ / 0.2M NH_4Cl Extractant.

To about 1800ml of deionized water add 35ml of conc. (10M) HCl, then 30ml of conc. NH_4OH (s.g. 0.91). Into this solution add 97.7g of $BaCl_2.2H_2O$ and adjust the pH to between 5 and 7 by addition of 1M HCl or NH_4OH. Make up to 2 litre volume with distilled water.

0.1M Ba/NH_4 Solution for performing dilutions

Add 1 litre of distilled water to 1 litre of 0.2M Ba/NH_4 solution

Primary Standards

Use commercially available 1000 mg/l standard solutions.

1 milliequivalent (me) of Ca = 20mg.	So:	Primary Ca Standard is 1000/20 **me/l.**
1 me of Mg = 12mg.		Primary Mg Standard is 1000/12 **me/l.**
1 me of K = 39mg.		Primary K Standard is 1000/39 **me/l.**
1 me of Na = 23mg.		Primary Na Standard is 1000/23 **me/l.**

Secondary Standards

Calcium (20 me/l)

Dilute Ca Primary Standard by adding 40 ml to a 100ml volumetric flask and make to volume with distilled water.

Magnesium (4 me/l)

This is a 2-step process. Add 12ml of Primary Standard to a 100ml volumetric flask and make up to volume with distilled water (= 10 me/l). Then add 40 ml of this solution to a 100ml volumetric flask and make up to volume with distilled water.

Potassium (4 me/l)

This is a 2-step process. Add 39 ml of Primary Standard to a 100ml volumetric flask and make up to volume with distilled water (= 10 me/l). Then add 40 ml of this solution to a 100ml volumetric flask and make up to volume with distilled water.

Sodium (4 me/l)

This is a 2-step process. Add 23 ml of Primary Standard to a 100ml volumetric flask and make to volume with distilled water (= 10 me/l). Then add 40 ml of this solution to a 100ml volumetric flask and make to volume with distilled water.

Working Standards

For each element, add 0, 1, 3, and 5 ml of Secondary Standard to 100ml volumetric flasks, then 50 ml of 0.2M Ba/NH_4 extracting solution, and make up to volume with distilled water. These standards should be stored in plastic bottles.

This gives:

- For Ca: 0, 0.2, 0.6, and 1.0 me/l Ca standards
- For Mg: 0, 0.04, 0.12, and 0.20 me/l Mg standards
- For K: 0, 0.04, 0.12, and 0.20 me/l K standards
- For Na: 0, 0.04. 0.12, and 0.20 me/l Na standards

Dilution of Extracts

Any dilution should be done with 0.1M Ba/NH_4 solution.

Use centrifuge tubes for dilution, and dilute in such a way that the final volume is 10 ml.

The usual dilution for extracts is:

- For Ca: (x5 dilution) 2 ml of extract + 8 ml of 0.1M Ba/NH_4
- For Mg: (x10 dilution) 1 ml of extract + 9 ml of 0.1M Ba/NH_4
- For K: (x5 dilution) 2 ml of extract + 8 ml of 0.1M Ba/NH_4
- For Na: No dilution

Note: To allow sufficient volume of standard solution during AAS analysis, add 20 ml to the centrifuge tubes. Thus, for Ca for instance, use 4 ml of extract + 16 ml of 0.1M Ba/NH_4.

Procedure

1. Add 2g of <2mm air dry soil to 50ml centrifuge tubes, followed by 10 ml of distilled water.
2. Place on the end/end shaker for 1 hour.
3. Record the pH (pH_{H2O}) and EC of the suspensions
4. Add 10 ml of 0.2M Ba/NH_4 extracting solution and return the tubes to the end/end shaker for 2 hours.
5. Centrifuge at 3000 rpm for 10 minutes (or longer if supernatant solution is not clear).
6. Pour supernatant solution into clean centrifuge tubes and retain for exchangeable cations analysis.

Calculation of Results

If AAS readout on the diluted extract sample = x me/L

Then concentration of element in original extract = DF . x me/L

= DF . x / 1000 me/ml

Extract Volume = 20 ml

Therefore amount of element extracted = 50. DF . x me.

Therefore concentration of element in soil = 50 . DF . x / 2g

= DF . x me / 100g.

eg. If AAS reading for Ca is 0.7 me/L, and extracted was diluted X5,

Then Ca in soil is 3.5 me/100g.

Determination of KCl Extractable Acidity (H + Al)

Reagents

1M KCl

Dissolve 74.6g of AR KCl and make up to 1 litre in distilled water

0.05M Sodium Hydroxide Solution

Use standard ampoule to prepare 1 litre of 0.5M NaOH solution

Add 100 ml of 1M solution to a 1000ml volumetric flask and make to volume with distilled water

Procedure

1. Add 4g of 2mm air dry soil to 50ml centrifuge tubes, followed by 40ml of 1M KCl solution
2. Place on end/end shaker for 1 hour
3. Centrifuge at 3000 rpm for 10 minutes
4. Take 25ml of supernatant solution and place in a 100ml beaker (with 25 ml of Water if desired). .
5. Titrate with 0.05M NaOH standard solution using phenolthalein solution for end point detection, or to pH 8.0 using a pH meter.
6. Obtain a blank value (for subtraction from all unknowns) by titrating 25ml of 1MKCl + 25ml water.

Results

Al in soil = 2 x (Titration – Blank) me/100g.

Note: 0.05M NaOH is 0.05 me/ml

So me Al in 25ml of extract = (Titration – Blank) . 0.05 me.

= (Titration – Blank) . 0.05 . 40/25 me/4g soil

= (Titration – Blank) . 0.05 . 40/25 . 100/40 me/100g soil

= (Titration – Blank) x 2.

Determination of Organic Carbon

Note: In this method of colorimetric determination, chromic (Cr^{3+}) produced in proportion to OC oxidised is measured. This means that the dichromate oxidising solution does not have to be standardized, as **residual** dichromate ($Cr_2O_7^{2-}$) is not being measured.

Reagents

0.5M Dichromate Solution

Dissolve approximately 149g of LR sodium dichromate ($Na_2Cr_2O_7.2H_2O$)) or approximately 147g of LR potassium dichromate ($K_2Cr_2O_7$) in 1 litre of distilled water.

Standard Solution

Note: 1 ml contains 5mg Carbon

Dissolve 11.8745g sucrose (previously dried for 24 hours over conc. H_2SO_4 in a desiccator) in water, transfer to a 1 litre volumetric flask, and make up to volume.

Procedure

Note: All conical beakers for standards and unknowns should be identical.

A set of sucrose standards must be run with each set of analyses to ensure that oxidising conditions fro standards and unknowns are identical.

1. Pipette 0, 2.0, 4.0, 6.0, and 8.0 ml of standard sucrose solution, corresponding to 0, 10, 20, 30, and 40mg C, into 125ml conical flasks. Place flasks in an oven no greater than 65^0C, evaporate to dryness and then cool. (Note: These working standards could be prepared the day before).

2. Weigh out finely ground (<0.5mm) air dry soil, 1.00g if expected OC content is <5% C and 0.20g if expected OC content is >5% C, and place in 125ml conical beakers.

3. Place all flasks on an insulating surface, add 10ml of dichromate solution, and swirl gently to ensure that all particles are wetted. Wait 10 minutes with occasional gentle swirling, and then carefully add 20ml of conc. H_2SO_4 with gentle swirling. Wait another 30 minutes with occasional swirling and then add 170ml of distilled water.

4. After the solutions have cooled, and particles have settled, determine absorbance at 600nm. Centrifuge any samples that are not clear before determining absorbance.

5. The Spectrophotometer will produce values as mg C. Therefore if 1g of soil has been digested, the Carbon content is Spectro value x 100 mgC/100g ie

   % Carbon (Walkley/Black) = Spectro value x 0.1

Determination of Available Phosphorus

(Bray 2-P)

Reagents

1. Extracting solution (or use existing extracting solution)

Dissolve 2.22 g ammonium fluoride A.R. (NH_4F) in distilled water and transfer to a 2 litre volumetric flask. Add 17 ml concentrated hydrochloric acid and bulk to volume with distilled water.

2. Mixed Reagent

(a) Molybdate Solution

Carefully add 250ml of conc. H_2SO_4 to 150 ml distilled water and allow to cool.

Dissolve 24g of ammonium molybdate in 100ml of distilled water and add this solution to the cooled sulphuric acid solution.

(b) Tartrate Solution

Dissolve 0.22g of potassium antimony tartrate in distilled water and make up to 100ml.

(c) Mixed Solution

Add 125ml of the molybdate solution to a 500ml volumetric flask, followed by 50 ml of tartrate solution, and make up to volume with distilled water. **This is the Mixed Reagent and should be kept in a refrigerator.**

3. Ascorbic Acid Solution

Dissolve 1.76g of ascorbic acid in distilled water and make up to about 100ml in a beaker. This solution should be prepared freshly on the day of analysis.

Standards

Primary P Standard 500 mg/l P

Dissolve 2.1968g KH_2PO_4 in distilled water and make up to 1 litre.

Secondary P Standard 10 mg/l P

Add 10 ml of 500 mg/l P to a 500ml volumetric flask and make up to volume

Working Standards 0 – 0.50 mg/l P

Add 0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml of 10 mg/l P to 100ml volumetric flasks. Make up to volume with distilled water to produce standards containing 0, 0.05, 0.10, 0.20, 0.30, 0.40, and 0.50 mg/l P.

Procedure

- Weigh 3g of <2mm air dry soil samples into 50ml plastic centrifuge tubes. Add 30 ml of extracting solution, apply caps to tubes, and shake vigorously for 45 seconds. Immediately centrifuge for 10 minutes at 3000 rpm.
- Pipette a 10ml aliquot of supernatant solution into a 50 ml centrifuge tube followed by 7 ml of distilled water, 2 ml of mixed reagent, and 1 ml of ascorbic acid solution. Screw on caps and shake well.

- Pipette 10 ml of each standard into centrifuge tubes, add 7 ml of distilled water, 2 ml of mixed reagent and 1 ml of ascorbic acid solution. Cap, and shake well.
- After 30 minutes, read absorbance at 882 nm.

Results

Phosphorus Content (mg/kg) = Spectro Value X 10.

Note:

If Spectro value = x mg P/L

= x /1000 mg P/ml

= x /1000 . 30 mg P / 3g soil

= x /1000 . 30 . 1000/3 mg P/kg soil

= 10.x mg P/kg.

Determination of Extractable Nitrate and Ammonium

(Using 2M KCl)

Reagents

2M KCl Extractant

Dissolve 149.1g KCl in distilled water and make up to 1 litre.

The Following Reagents are for Nitrate Determination

Copper Solution

Dissolve 2g $CuSO_4.5H_2O$ in 500ml of distilled water

Dilute 3ml of this solution to 500ml as a **Working Copper Solution**

Hydrazine Sulfate Solution

Dissolve 0.3g in 500 ml of distilled water. This solution is stable for about 1 month.

Buffer Solution

Dissolve 11g of sodium tetraborate and 1.25g of sodium hydroxide in about 450 ml of distilled water and make up to 500 ml.

Colour Reagent

Add 50 ml of conc. HCl to about 400 ml of distilled water. Dilute 5g of sulphanilamide in this dilute acid. Then add 0.25g of N-1-naphthyldiammine dihydrochloride and when dissolved (with stirring) make up to 500 ml.

Standards for Nitrate Determination

Primary Standard 100 mg/l N in 2M KCl

Dissolve 0.3609g of oven dried potassium nitrate in about 200 ml of distilled water in a 500ml volumetric flask. Add 75g of KCl and make up to volume with distilled water.

Working Standards

Pipette 0, 1.0, 2.0, 3.0, and 4.0 ml of primary standard into 100ml volumetric flasks, and make up to volume with 2M KCl solution.

Procedure for Extracting Nitrate and Ammonium

Place 4g of <2mm air dry soil samples in 50 ml plastic centrifuge tubes, followed by 40ml of 2M KCl extractant. Place racks on the end/end shaker for 1 hour.

Centrifuge at 3000 rpm for 10 minutes.

Nitrate Determination

For colour development of standards and unknowns:

- Add 3 ml of working copper solution to a 50ml centrifuge tube
- Add 1.5 ml of **standard or unknown** and swirl
- Add 2 ml of hydrazine solution and swirl
- Add 3 ml of buffer and swirl

Place in a 37^0C oven for 15 minutes

- Add 3 ml of colour reagent and swirl

Allow at least 15 minutes for full colour development and then read at 520 nm.

(The colour remains stable for at least 12 hours)

Ammonium Determination with Markham Still

- Check that the 3-way valve is allowing steam to vent to waste
- Ensure water is flowing through the condenser
- Place a 50ml beaker containing 3ml of boric acid under the condenser outlet
- Place 5ml of supernatant solution in the Determination Flask, apply stopper and affix to the Markham Still.
- Add 5ml of NaOH solution to the plugged inlet well.
- Slowly lift the plug to allow the NaOH to enter the still, but maintaining a liquid seal
- Turn the 3-way valve to allow steam to enter the Determination Flask, ensuring that the tip of the condenser is immersed in the boric acid.
- Continue the distillation until about 25 ml of condensate has been collected
- Remove the 50 ml beaker and divert steam to waste.

This procedure should also be followed using only 2M KCl extract to act as a blank.

- Titrate the condensate with 0.01M HCl.

If: Volume of Extract = V_1 and Vol. added to the still = V_2, then $V_2/V_1 = 8$

% NH_4-N = (Titre-Blank) x 0.14 x V_1 / V_2 x 1/1000 x 100/soil wt

= 0.0035 x (Titre-Blank) x V_1 **/** V_2

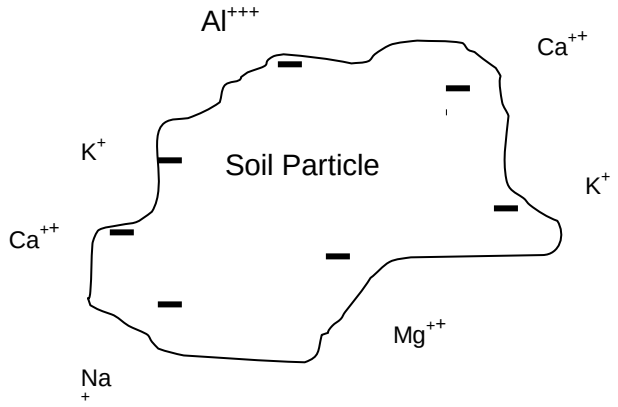
= 0.028 x (Titre-Blank) when 5ml of extract is added to the still.

ADDENDUM

1. Why is it important to determine the exchangeable cation content of soil?

Certain particles in soil (clay and organic matter) have **negative charge** on their surfaces that is balanced by **positively charged** ions (cations) that surround the particle. These cations can be exchanged or replaced by other cations in the soil water, and so are called **exchangeable cations**.

The surface negative charge is referred to as the soil **Cation Exchange Capacity** (CEC) and the principal exchangeable cations are Calcium **(Ca^{++}),** Magnesium **(Mg^{++}),** Potassium (**K^{+}),** Sodium (**Na^{+}),** and Aluminium (**Al^{+++}**).



Three of these cations are **Macronutrients**, i.e. they are essential for plant growth. They are Calcium, Magnesium, and Potassium. Sodium has very limited value as a plant nutrient, but can greatly affect soil physical properties if present in sufficient amount. Aluminium can be toxic to plants and can also cause soil acidity if present in sufficient amounts.

There are a number of ways of determining CEC, ie. the maximum amount of cations that can be held in the soil in exchangeable form. In many cases a good measure of CEC is the sum of exchangeable cations. **CEC = Ca^{++} + Mg^{++} + K^{+} + Na^{+} Al^{+++}.**

Once we determine CEC, as well as the Exchangeable Cations, we can examine several aspects of the date to determine the 'health' of a soil e.g.

1. Actual amounts of Ca, Mg, and K for plant nutrient supply
2. Balance of Ca: Mg; K
3. Na as a percentage of CEC for soil physical limitations
4. Al as percentage of CEC for acidity and plant toxicity considerations.

2. Some Good Laboratory Practices

Because relatively salty solutions (0.1M Ba/NH_4 Cl) are being aspirated during cation analysis using the AAS, aspirate with water for at least 5 seconds between samples. At the completion of the run, aspirate with 20% HCl for 1-2 minutes, then with water.

At the completion of a UV/Vis Spectro run, immediately empty the cuvettes, wash with water, and soak overnight in 2% Decon 90 solution. Then rinse with tap water, followed by distilled water.

At the end of each week, remove the buckets and adaptors from the centrifuge and wash with water. Invert and allow to dry before re-assembling on the centrifuge rotor.

When using the dispensing pipettes, apply a gentle action so that solution is not sucked up into the barrel of the dispenser.

After completion of a determination using centrifuge tubes in racks, use vortex mixer to re-suspend the soil and discard into a bucket. Wash the tubes with water and then soak overnight in Decon 90. Then rinse with tap water followed by distilled water.