Laboratory Methods of Soil and Plant Analysis: A Working Manual

Second Edition

J.Robert Okalebo, Kenneth W. Gathua

and Paul L. Woomer

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The Second Edition

by

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"To the Soil Chemistry Technicians at the National Agricultural Research Centre, Muguga, Kenya, without whose dedicated efforts this book would not have been possible."

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A Working Manual

The Second Edition

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Laboratory Methods of Soil and Plant Analysis

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INTRODUCTION TO THE SECOND EDITION

Analyzing the chemical characteristics of soils and plants in a reliable, broadly applicable, cost-and-time effective manner is of great importance to the agricultural, environmental and development communities in Africa. The above statement was applicable to the original publication of *"Laboratory Methods of Soil and Plant Analysis: A Working Manual"* in 1993 but has become even more important at present because of several crucial scientific developments over the past decade.

Soil nutrient depletion in smallhold farming systems became recognized as a causal force leading to chronic food insecurity and rural poverty in Africa (Smaling *et al*., 1996, 1997). This awareness led to greater emphasis upon examining nutrient cycles and budgets at scales ranging from the farm to the regional levels. This information in turn contributed to our abilities to develop and implement strategies for soil fertility replenishment. One such strategy is summarized as *"P from the bag and N from the air"*, where the nutritional constraints of symbiotic nitrogen fixing legumes is overcome, allowing for greater inputs of atmospheric nitrogen (Sanchez *et al*., 1997). Specific mechanisms for phosphorus replenishment using indigenous deposits of phosphate rock that is mobilized by development agencies and the private sector were advanced (Mokwunye and Bationo, 2002; Woomer *et al*., 1997a). Others advocate that Africa's food security may only be achieved through the adoption of high yielding technologies brought about from an African "fertilizer-based green revolution" as has occurred elsewhere in the tropics (Quinones *et al*., 1997).

A more ecological approach to nutrient management has also emerged over the past decade known as Integrated Nutrient Management (INM). Jenssen (1993) defined INM as involving *"… the combined use of organic and mineral fertilizers in such a way that the required nutrients are applied and the soil organic matter content is maintained"*. Others have expanded upon this principle to include biological nitrogen fixation, and identified specific interventions within different farm enterprises available to smallholders (Wortmann and Kaizzi, 1997; Woomer *et al*., 1999). INM and its more holistic counterpart, Integrated Natural Resource Management, represent a paradigm shift from focus upon commodities and inputs to a wider understanding of agricultural ecology as interactions between different rural enterprises. Integrated management approaches are less agronomic and more environmental, consisting of systems studies rather than field experiments. This approach leads not to top-down prescription of technical "solutions", but rather to greater capacity of stakeholders to participate in adaptive learning.

Another recent development is the acceptance by the scientific community that human activities have affected the earth's atmospheric composition and this in turn has altered the climate, often with disastrous impacts. Carbon dioxide and other "greenhouse gasses" are causing the earth's temperatures to rise and resulting in less reliable rainfall patterns. Ten years ago, agriculturalists felt little connection to the "futuristic" concerns of global ecologists, but today it is widely accepted that the conversion of natural ecosystems to agriculture and subsequent management of soils is the second leading contributor to global atmospheric change (after the combustion of fossil fuels, see Bouwman, 1990). Furthermore, tremendous potential exists to mitigate climate change by storing larger amounts of carbon as biomass and soil organic matter within agricultural systems and developed nations stand ready to pay land managers in developing countries to help combat climate change (Wisniewski and Sampson, 1993; Lal *et al*., 2000). This is a profound development that will affect the actions of the agricultural community for the next several decades, and scientists must be prepared to document the carbon sequestration resulting from different land management and restoration options (Lal *et al*., 2001; Woomer *et al*., 1997b).

Other recent developments signal the need to better analyze the chemical compositions of plants and soil. Plant breeders no longer select crops based upon yield properties alone, but rather recognize the importance of nutrient use efficiency and tolerance to nutrient stress (DeVries and Toenniessen, 2001).

Soil biology is now sufficiently developed that litter decomposition and nutrient mineralization operate in a more predictable manner (Woomer and Swift, 1994) allowing the benefits from organic inputs to be better managed (Mafungoya *et al*., 1997; Palm *et al*., 2001; Giller 2002). Computer simulation models have become an important tool for the integration and extrapolation of research results, but these models require careful initialization and validation with carefully collected data before the outputs obtain credibility (Parton *et al*., 1994). Even the forces guiding economic and policy reforms affect the manner that plant and soils investigations are being conducted (Adipala *et al*., 2001). The drive towards greater decentralization of agricultural services requires greater capacity at local levels and those samples be collected, processed and transported in a manner that preserves their integrity.

Readers may be asking themselves at this point what does this review of recent developments in nutrient and carbon management have to do with a handbook describing methods of plant and soil analysis? Our reply: all of the developments over the past year place greater importance on national and public university laboratories to assist Africa to better manage its natural and agricultural resources, and this in turn requires that the chemical properties of plant tissues and soils be accurately quantified in ways that are comparable between locations and over time. To better promote these goals is our motivation in producing the second edition of *"Laboratory Methods of Soil and Plant Analysis: A Working Manual"*, a task that we were encouraged to undertake by many members of the Soil Science Society of East Africa and the African Crop Science Society over the past several years.

The production of this second edition of our manual follows several considerations. First, we recognize and apologize for some technical and production errors for specific methodologies from the original edition published in 1993. We are very grateful to the users and readers of the first edition who have pointed out the specific errors and suggested relevant corrections. We note the existence and usefulness of several other manuals of soil and plant analysis but we are also sensitive to the working conditions in many inadequately equipped laboratories in the third world, and we adjust our methods whenever possible to those disadvantageous conditions. We focus on methodologies that exclude the use of costly equipment and reduce the requirements for chemicals and other consumables such as filter paper. Furthermore, useful feedback from those who relied upon the original edition has led to expanded scope of this manual to include additional methodologies for soil physical, chemical and biological analyses. We have also expanded the interpretation of analytical data interpretation section as the basis for soil and plant analysis.

The original edition of this laboratory manual was developed as a component of a workshop on analytical methods held at the Kawanda Agricultural Research Station, Uganda during February 1993. The workshop was a collaborative activity between the Kawanda Station and KARI-Muguga, UNESCO's Tropical Soil Biology and Fertility Programme and the Soil Science Department of Makerere University. That Kawanda workshop and the original publication of the manual were funded through a grant from The Rockefeller Foundation. Again, The Rockefeller Foundation has approved funds that allow for the authors to revise and publish this manual, for which we extend their sincere gratitude. We also acknowledge the contributions by colleagues on laboratory methods. These workers include J.M. Anderson, M.A. Beck, H.F. Birch, C.T. Figuereido, M.T. Friend, P.K. Garberg, J.S.I. Ingram, S.K. Kimani, N.G. K'ungu, J.K. Lekasi, N. Mangale, A.O. Moshi, , F.M. Mwaura, J.F. Osborne, C.A. Palm, P.K. Patel, J.L. Pleysier, J.B.D. Robinson, P.M. Rugui,.R.J. Scholes, J.S. Tenywa, M.M. Tenywa and J. Wendt. Dr. Paul Smithson provided the authors with a thorough review of the original edition, identifying and correcting several incomplete or inaccurate procedures. Finally, we are grateful to the members of the Soil Science Society of East Africa, the African Crop Science Society and the Forum on Agricultural Resource Husbandry for their encouragement toward the production of this manual.

J.Robert Okalebo, Kenneth W. Gathua and Paul L. Woomer August 2002

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CHAPTER 1

THE LABORATORY AS A WORKPLACE

The following should be taken into consideration in order to maintain a safe, efficient and enjoyable working environment within the analytical laboratory:

Laboratory Design. The manager of an analytical laboratory should develop a system which maximises safety, reproducibility and through flow of samples. These objectives are accomplished by creating a work environment, which is efficient from both the ergonomic and the economic perspective. In general, the samples should flow smoothly from one analytical step to the next without being carried about unnecessarily. This is basically a four-step process, beginning in the area where the samples are registered and prepared for analysis, proceeding through a reception workstation where samples are processed for measurement, continuing to areas where sample measurements are conducted and concluding in a cleaning station where wastes are disposed and reusable supplies recovered.

Whenever possible, the different analytical work stations should be assigned permanent, designated positions within the laboratory, allowing for the specialised needs of a given procedure to be kept close at hand. Other facilities that promote smooth laboratory performance include:

1. A separate room for receiving and recording of incoming samples in order to avoid contamination.
2. Facilities for sample drying, grinding, sieving and storage. This area can be close to the sample reception room, but again must not pose a contamination threat to the remainder of the laboratory.
3. Vibration-free benches, on which analytical balances and delicate instrument are placed.
4. Lockable rooms and/ or cabinets for the safe storage of chemicals, possibly separate rooms for organic and inorganic chemicals. Inflammable organic chemicals must always be stored in fire-proof cabinets. A running inventory of available reagents and their use must be maintained.
5. Laboratory instruments must be placed in special rooms, possibly air conditioned, dust free and should be away from the rest of the laboratory space in order to maximise their run time.
6. Well-defined area for disposing of laboratory wastes, washing and drying glassware. The reagent used in one procedure must not become a contaminant in the next!
7. Designated area for staff breaks

Safety in the Workplace. The most important component of any laboratory is its human resources, yet a laboratory, by its very nature, cannot be regarded as a safe place. Because of this contradiction, laboratory safety procedures are essential to its operations. Most of the laboratory procedures involve manipulation of hazardous substances, it is necessary that ready access to protective and first aid supplies be provided. These include protective eye ware, laboratory coats, chemical and heat resistant gloves, tongs, eye wash solution, emergency showers and first aid kits containing antibiotic cream, burn medicine and bandages. The protective items that are openly displayed are far more likely to find regular use than those kept away from sight and will remain in a sanitary condition when centrally located in glass-fronted cabinets. The following are additional guidelines that serve to maximise safety within the laboratory:

1. Fire fighting equipment must be readily accessible in the event of fire. Periodic maintenance inspections must be conducted.
2. First-aid supplies are a necessity and laboratory staff should be well trained in their use. Replacement of expended supplies must take place in a timely fashion.
3. Only those reagents and items needed for a given procedure should be in work areas. Reagent and back-up supplies should be kept in stores and not in working areas.

4. All reagent bottles should be clearly labelled and must include information on any particular hazard. This applies particularly to poisonous, corrosive and inflammable substances.
5. Procedures involving strong acid and/or high temperatures must be conducted within a fume hood.
6. Chemical spills should be cleaned promptly and all waste bins regularly emptied.
7. Work areas should be kept "clutter-free" with a clean water supply and ready access to cleaning equipment.
8. Liquid wastes should be poured carefully down a sink with sufficient water to dilute and flush them away. Where possible a dilution tank can be installed between the sinks and main disposal site. This is because many analytical procedures produce toxic and acid wastes. Keep in mind that local ordinances often prohibit the disposal of specific substances through the public sewage system therefore safe disposal must be sort. Solids must not be placed in sinks in order to avoid blockage of drains.
9. Eating, drinking and smoking should be discouraged at all times from the laboratory for both reasons of health and to avoid contamination. Eating and drinking should be confined to designated areas during staff breaks. Those staff who smokes should be required to pursue their habit outside of the laboratory.

Personal Habits Protect Safety! As stated above, eating and drinking within the laboratory should be limited to designated places and smoking should be prohibited, but several other personal habits can greatly influence safety within the laboratory:

1. Hands should be thoroughly washed when entering and leaving the laboratory and before and after designated breaks.
2. Testing chemicals by taste or odour is extremely dangerous and should be forbidden.
3. Personal items such as coats, hats, umbrellas and handbags should be kept in shelves or lockers away from the workstations.
4. Persons with long hair should be required to tie it into a ponytail and/or cover with a cap. Long beards should be discouraged but, when considered necessary (e.g. through ethnic belief), should be frequently washed and combed away from the laboratory.
5. Drinking water should be kept and consumed away from laboratory workstations, and whenever possible, foot operated drinking fountains should be provided for the use by staff in designated areas.
6. Disposable paper cleansing tissue rather than cloth handkerchiefs should be used when necessary for personal purposes.
7. High-heeled shoes and open sandals should not be worn in the laboratory by those staff working with dangerous substances or sharp instruments

Laboratory Bench Surfaces. Laboratory benches should be surfaced with heat resistant, non-absorbent material such as Formica. Many older laboratories have wooden benches that should be protected by promptly cleaning chemical spills. Disposable plastic sheeting may also be placed on the top surfaces of wooden benches. The obvious objective of preserving laboratory bench surfaces is to reduce risk of contamination. Similarly, locally constructed fume hoods common in many developing countries should be lined with corrosion and heat resistant material.

Electrical Supply and Reliability. Most analytical procedures involve the use of electrical equipment and instruments.. The reliability of the electricity supply is of great importance in the preventative maintenance of laboratory instruments. The impacts of electrical surges, oscillations and blackouts are common in developing countries, but their negative effects can be minimized by installing surge-protected plugs and stabilising the power supply. As most equipment operates on low wattage, an un-interruptible power supply (UPS) provides stable power and allows the completion of batch measurements in the event of power outage.

Water Supply and Quality. Water is not only the essence of life but the wellspring of laboratory performance. The effects of short-term interruptions of water supply can be minimized by providing each laboratory with reservoir tanks. Placement of this tank on the laboratory roof or near the ceiling provides sufficient water pressure for most laboratory purposes. Water deionizing and distillation facilities are an essential feature of any analytical laboratory. For most analytical purposes, single distilled or deionised water can be used. Some critical analyses must be conducted with water that has been deionized then distilled (or even double distilled).

Disposal and Cleaning Procedures. Disposal of laboratory wastes and the washing of reusable laboratory supplies is the least existing but most important chore within any laboratory. The lifespan of laboratory glassware, and especially plasticware, is extended by emptying and washing immediately after use. If this is not possible, emptied, rinsed but unwashed materials should be soaked in water until they can be washed. Short-term accumulation of un-cleaned laboratory materials is inevitable so laboratory priorities must be readjusted on an *ad hoc* basis before this accumulation begins to interfere with laboratory performance. Those washing glassware must be aware that wash and rinse water may contain harmful substances and, as a general precaution, wear protective gloves. In general, washing of laboratory supplies falls into two categories; superficial de-contamination *vs* cleaning surfaces of residues which are difficult to remove. Superficially dirty glassware is cleaned by washing with a brush in hot water containing a soap or detergent and then rinsing with tap water and later with distilled water. Grossly contaminated or stained glassware require more specialised cleaning procedures:

1. Stained glassware may be cleaned by a solution of ferrous sulphate in dilute sulphuric acid. Permanganate stains may be effectively removed by sulphurous acid. Iron stains can be removed by 1:1 HCl acid. Deposits of precipitating "chalk" that adhere to the sides of vessels are easily removed by washing with dilute hydrochloric acid.
2. Chromic acid cleaning mixture is especially effective in removing stubborn contamination. This is prepared by dissolving 25 g sodium dichromate in 25 ml of water and increasing the volume to 1 litre by carefully adding concentrated sulphuric acid. Small amounts of this are added to the insides of contaminated glassware and then rinsed away prior to regular washing.
3. Another effective pre-treatment is a cold solution mixture of 5% hydrofluoric acid, 33% nitric acid, 2% Teepol and 60% water

Contamination. The most insidious enemy of any analytical laboratory is contamination resulting in analytical errors and, therefore, its sources must be identified and eliminated. Some of the common sources are:

1. External dust blown from the surrounding environment.
2. Internal dust resulting from cleaning operations.
3. Cross-contamination deriving from handling many samples at the same time.
4. Failure to store volatile reagents well away from samples.
5. Washing materials, particularly soap scouring powder.
6. Smoking in the laboratory.

Levels of Accuracy. Different steps in an analytical procedure often require that materials be measured at different levels of accuracy. In general, standard solutions must be prepared using the maximum levels of accuracy available within the laboratory. For example, when preparing a 1000-ppm K solution one must weigh 1.9069 g of KCl of analytical grade reagent and fill to 1000 cm^3 in a volumetric flask rather than a graduated cylinder. On the other hand, a 1.0-g soil sample need only be weighed to 2 significant decimal places (± 0.005 g). Those reagents required in excess are prepared at the lowest levels of accuracy. These are often described as requiring "%" solutions.

Batches. For efficiency in the management of samples within a laboratory, it is important to have the samples assigned to groups or batches. This especially applies when handling large numbers of

incoming samples. Naturally, the batch size is dictated by the maximum number of samples to be analyzed during a single working session. In principle, the larger the batch size, the more time elapses between measurement of the first and last samples within a given batch. Care must be taken to avoid working with excessively large batch sizes especially in cases where the chemical reactions being used as indicators continue with time. In such cases a sample value which accurately compares to a standard value from the beginning of a batch may not compare well if it is measured toward the end of an excessively large batch. All batches must include blanks, repeats and reference samples in order to optimise quality control.

Record Keeping. To err may be human but to lose data after careful experimental preparation and laboratory analysis is unforgivable. Subordinates caught red handed in the act of recording important data on small scraps of paper must not be forced, despite all first instincts, to eat that paper after carefully recopying the results in their proper place (remember our ban on eating within the laboratory) but rather must be reminded of laboratory record keeping procedures . Data must be recorded in specific laboratory record books which contain all the data pertaining to a given analysis including, for example, sample identity, weight, final volume or absorbency. The records which contain primary data should not leave the laboratory (at least no farther than the nearest computer). Measurements of blanks and standards should always be recorded together with the sample readings for that particular batch. Reports should be produced in duplicate with one copy given to the individual who submitted the samples for analysis and another copy filed by the laboratory manager.

The Laboratory as a Challenging Work Environment. Working within an analytical laboratory can be either challenging, rewarding and fun or a dull, tedious and confusing existence depending on individual attitudes and how well a laboratory is managed. A common ingredient in all successful laboratories is the feeling that everyone is an important part of a team of co-workers, not just the supervisor or dishwasher or whatever. No effective manager can consider themselves above performing any laboratory chore when necessary. Staff members who demonstrate special initiative or interest in additional activities should be encouraged regardless of their position within the laboratory. Nonetheless, the work must be done and everyone should be aware of the role within the team.

The Laboratory as a Social Setting. In our experience, the best performing laboratories are those that hold regular staff meetings and arrange for periodic social gatherings. Too often, staff meetings are only held after something has gone wrong within the laboratory, causing an awkward situation. A better approach is to hold regular meetings where issues are discussed in an open manner before something goes wrong. The Laboratory Manager should also compile some performance indicators that are shared with the group and outstanding efforts by staff members should be acknowledged. Social gatherings, such as an annual picnic or staff party before a major holiday, bring staff together in an informal setting that results in a group identity and a spirit of teamwork that cannot be achieved through any other means. These events should be planned well in advance and both the staff and management should be expected to contribute to them. The retirement of a colleague is an important, and somewhat sad, event and plans should be made accordingly.

CHAPTER 2

QUALITY CONTROL AND STANDARDIZATION PROCEDURES

Quality control is an essential part of sound laboratory practice, especially during routine analyses where gradual drift may occur as a result of contamination, changes in reagent quality, environmental differences, operator error and instrument calibration or failure. Maximum reproducibility and adequate accuracy of results are important objectives. Repeated measurement of an air-dried sample should provide consistent results when analyzed over time for most routine chemical procedures. The deviation of an observed value from its absolute ("true") value results from either systematic or random errors. Once identified, systematic errors are more easily corrected than those, which occur at random. Three precautions are essential to laboratory quality control and should be routinely included among the test samples. These precautions are the use of blanks, repeats and internal references and are described later in this section.

Laboratory precision requires that the analysis of identical samples yield near-identical results. Accuracy requires that the overall mean of several results fall close to a known "true" value. An analogy of precision and accuracy between laboratory practice and archery is presented in Figure 2.1. The results obtained in Case 1 is neither precise nor accurate and, while reliable results may be obtained on occasion, we cannot begin to improve the results because precision and accuracy are hopelessly confounded. The results in Case 2 are precise but inaccurate because of systematic errors which causes results to drift toward the upper right (or "true" value). The overall mean of Case 3 is accurate, but no single result is acceptable because of the imprecision. The results of Case 4 are both precise and accurate. In Case 4, relatively few results provide a reliable measure of the "true" value but only after both precision and accuracy have been assessed.

Whenever a new procedure is introduced within the laboratory, it is important that both precision and accuracy be evaluated and that these be compared to current results. For example, if a candidate alternative procedure is identified for introduction as a routine laboratory analysis on the basis of its time or materials efficiency, this method must be comprehensively tested against those already in use. First, both methods should be compared for a homogeneous test sample using ten-fold replicates and the standard deviation calculated. This provides a measure of precision. Then a known amount of reagent should be added to the homogeneous test sample, the procedures repeated and the mean and standard deviation calculated. The agreement between the increase in the values obtained to the known increase in test sample concentration provides a test of accuracy. For procedures in which the test material is known to interact with the added reagent, as with phosphorus sorptive soils, conduct this test with reagent solutions.

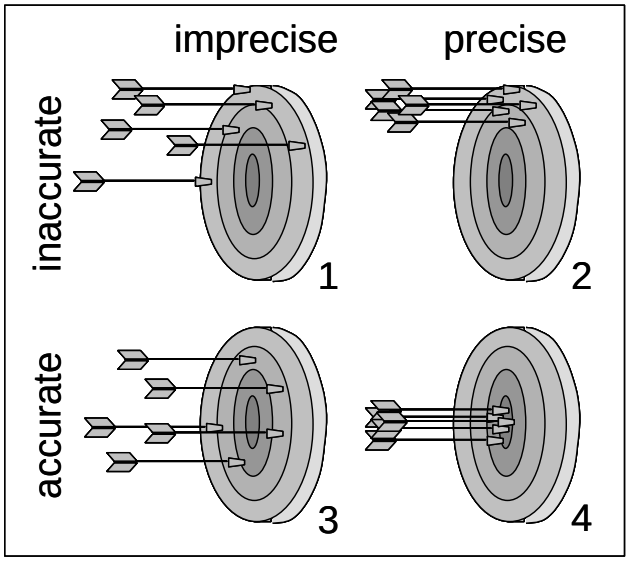

Figure 2.1. Assessment of precision and accuracy are prerequisites in laboratory quality control as illustrated by the analogy between analytical results and archery.

Blanks. Blanks are reaction vessels that are subjected to identical procedures as the samples in a given batch but have no added test material. Blanks allow correction for any background contamination introduced from reagents, filter papers or other systemic sources of error. Provided the blank values are consistent, the mean value can be subtracted from the sample result. When blanks yield large values, this suggests excess extraneous contamination and when observed, requires that an entire batch analysis be repeated.

Repeats. At least 1 in 10 samples selected from the test materials and placed at random within the batch should be analyzed in duplicate. The choice of 1 in 10 is a suggested compromise between the ideal of analyzing all samples in duplicate and the time, effort and expense of doing so. Obviously, the analytical results for given pairs of duplicate repeats should closely resemble one another. In general, repeat values should fall within ± 2.5-5.0% of their mean depending on the analysis in question and any greater discrepancy must be investigated. If repeat values are not consistent then the entire batch should be re-analyzed.

Internal References.
Internal reference samples are necessary for each type of test material and analysis practiced within the laboratory. The internal sample should not be the same as the homogenous material routinely used in the testing of new methods and analytical technique. A sample obtained from a large, well mixed and homogeneous bulk composite should be included in each batch as an internal reference. The values obtained for this sample should be the same for each batch analyzed and variation from the mean as calculated over previous batches indicate an error. Plot analytical result for the internal reference on a quality control chart (Figure 2.2) to monitor the performance of the analyses over time. Take corrective action if a single value exceeds the ± 3 standard deviation limits or if two successive values exceed the ± 2 standard deviations. Periodically reassess the critical limits by recalculation of the overall standard deviation of the internal reference sample as more data are accumulated.

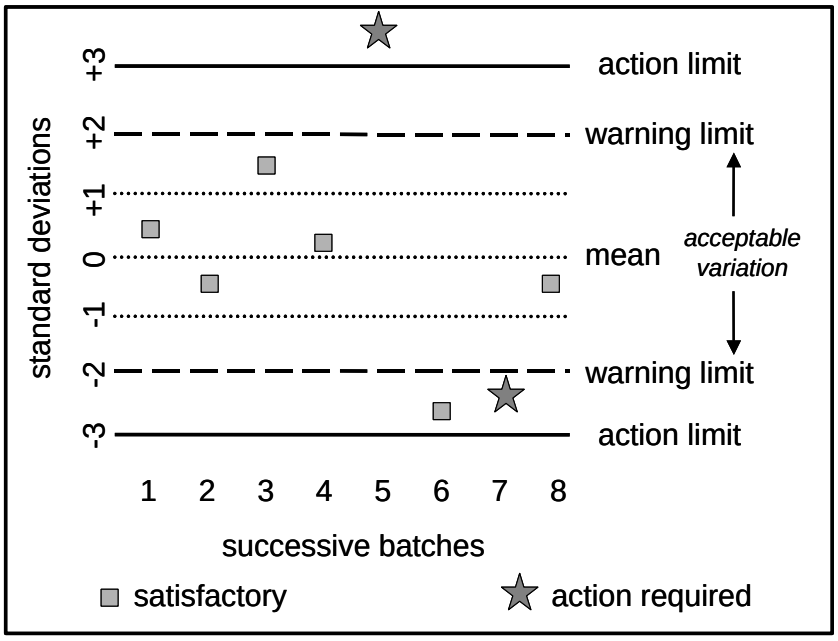

Figure 2.2. Quality control chart to compare sequential measurement of an internal standard to the critical action limits (after Ingram and Anderson, 1993).

Standardization of Methods. Results can only be validly compared to one another when these have been obtained using standardised methods. Collaboration between laboratories can be improved by exchanging reference materials and then comparing their results. Such materials are referred as "external references". The preparation of this book is intended to assist in the standardization of methods between soils analytical laboratories in East and Southern Africa.

CHAPTER 3

SOIL AND PLANT SAMPLING

A soil or plant tissue analysis and interpretation is only as accurate as the sample represents the phenomenon that is intended for measurement. Soil and plant laboratory analytical data are the result of a number of successive manipulations each with its own controlled and un-controlled sources of error and care must be taken at each step to insure that controlled errors are prevented and un-controlled error minimized. A soil sample is recovered from the field as a "discrete" sample, mixed, dried, ground and sieved, stored, divided into sub-samples, extracted, measured and analytical data calculated. Similarly, a plant sample is obtained, washed, chopped, dried, ground, stored, digested, measured and also calculated. Any of these operations is subject to errors, so that the final analytical results may deviate from the accurate value. In reality, the analytical results for apparently identical samples often show large variations, which are due to sampling and handling rather than measurements within the laboratory (Okalebo, 1985; Houba *et al*., 1990).

It is essential to know the conditions for representative sampling and to recognize the possible pitfalls involved. These pitfalls relate to the sampling technique that is the manner that individual samples are obtained, and the sampling strategy, which is the location and number of samples. It is important that the sample reflects the properties of the larger system of interest. In addition, sampling procedures are different for different sorts of investigations. For example, soil samples designed to characterize soil fertility within different farm enterprises will require that several sub-samples from the plant root zone serve as a composite from each enterprise, whereas soil samples intended to characterize the process of nutrient leaching or soil pollution will require that numerous soil depths are sampled from fewer locations within the farm.

Soil sample heterogeneity is caused by variations in soil parent material and the process of soil formation, leaching of organic and inorganic materials, biological activities in the soil and volatilization from soils (Houba *et al*., 1990). Influences of human activities, such as tillage and fertilization history, may also contribute to sample heterogeneity. The representativeness of a soil sample may also be influenced by the uniformity of the composite sample, seasonal variations and changes in the sample during processing and storage.

Soil Sampling Strategies. The above factors demonstrate clearly the difficulty of taking a representative sample for an area or from a soil profile. There is no standard procedure applicable to all soils and conditions. However, points to keep in mind when sampling soils are:

1. Series of cores, taken according to some systematic grid layout of the area, should be composted.
2. Separate soil cores or replicate sets of composite samples should be analyzed to determine statistical significance of results of the final composite sample.
3. The number of soil cores to be composted depends on the heterogeneity of the soil, the degree of precision desired, and the element to be determined.
4. Cultivated soils are generally less homogeneous than virgin soils.
5. The need for taking separate composite samples in order to represent different horizons of soil profiles or root zones or parts of the area, belonging to different soil types.
6. Contamination by crop residues, manure, fertilizers, must be avoided.

As indicated above, several soil sampling procedures and patterns are used for agricultural purposes. For example, in the Netherlands, a minimum of 40 cores are taken on arable land area of 2 hectares to 30cm depth for fertilizer recommendations, apart from the mobile and highly variable

Table 3.1. Relationship between the coefficient of variation of chemical analysis and the number of soil cores taken (after Houba *et al*., 1990)

Number of Soil Cores	Resulting Coefficient of Variation (%)
5 cores	40%
10 cores	28%
20 cores	23%
30 cores	20%
40 cores	17%
50 cores	15%
100 cores	9%
150 cores	8%

N, with variations occurring with seasons down the soil profile. But for the grasslands with extensive rooting systems and organic matter accumulations in the surface, 50-60 cores are taken to 5cm depth (Houba *et. al*., 1990). In Kenya, soil-sampling procedures vary among institutions. Thus the National Agricultural Research Centre (NARC), Kenya Agricultural Research Institute (KARI) and Moi University, Eldoret, take their surface soil samples to 15 and 20 cm depths. Moreover, high organic matter and major nutrient levels are contained in these plough depth soils. In surveys to diagnose nutrient deficiencies and variability within and across smallholder cropland and soils, a minimum of 9 core samples for about 0.5 ha fields were found to be adequate to detect differences in nutrient status of soils in Eastern, Central and Western Kenya (Okalebo *et. al*., 1992; Woomer *et. al*., 1999).

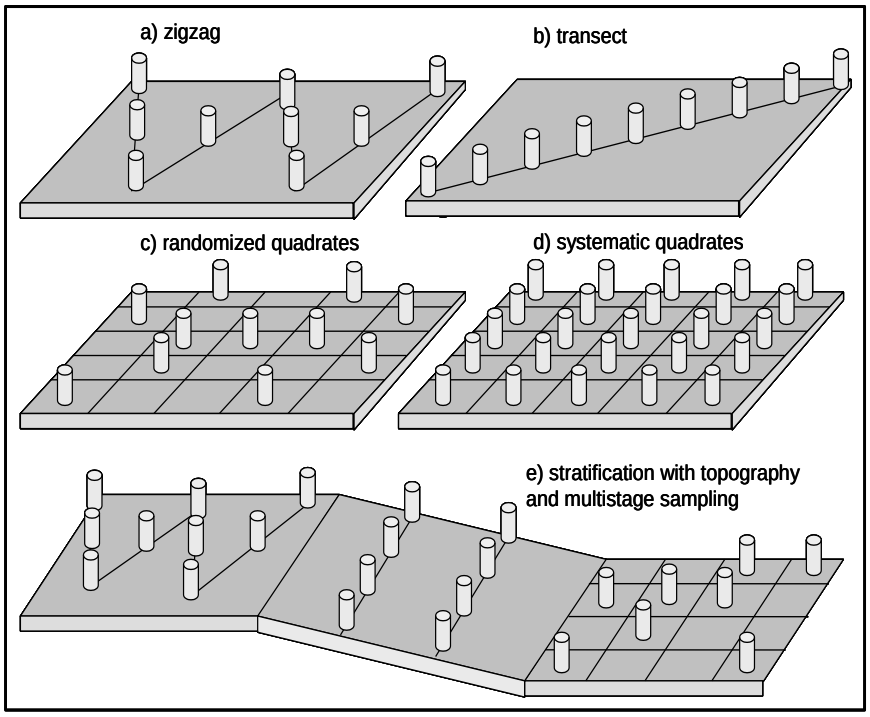

Figure 3.1 Diagrams of different sampling approaches for agricultural lands that include (a) zigzag pattern, (b) along a transect, (c) random sampling within quadrates, (d) systematic sampling within quadrates, (e) stratification by topography and multistage sampling.

Elsewhere, Colwell (1971) reported that at least 9 to 12 cores should be taken from experimental sites for areas of about 0.4 ha in New South Wales, Australia, to detect differences in soil properties. The overall number of cores to be taken is based on an acceptable Coefficient of Variation for the soil characteristics. This coefficient of variation of analysis varies with the number of cores taken (Table 3.1). Thus low coefficients of variation are associated with large numbers of cores taken. Soil sampling is often done at the beginning of cropping seasons (prior to treatment additions) to characterise sites or to obtain the nutrient status and their variability across fields. Sampling may also be executed during the growth cycle of the crops. Date obtained is used to assess fixation of nutrients (mainly P), N release and leaching from treatments and so forth.

In the sampling procedures the cores are taken evenly across the field, avoiding irregularities and borders of the field, in patterns given in Figure 3.1. The sampling patterns may deviate from the ones sketched above, but it is important to note that the relevant or selected sampling procedure will depend on the target of research or activity (such as commercial soil testing), the labour costs, types and costs of analysis. Other soil handling and preparation steps prior to analysis are described separately under each method of analysis.

Plant Sampling Strategies. Variability in nutrient concentrations in plant tissue is dependent on the plant and its species, plant part sampled and the age of the plant. For example, phosphorus is accumulated in high concentrations in young growing parts of a plant and in the seeds and fruits. These guidelines are used in relation to plant sampling (see Chapter 22 that focuses on the maize, banana, beans, citrus and cut flowers that are of fundamental importance to food security and cash flow to most developing countries). Details for sampling procedures for other plants/crops/trees are given in a range of texts (e.g. an Interpretation Manual (eds. D.J. Reuter and J.B. Robinson, Inkata Press, Melbourne, Sydney, Australia, 1986). In summary, careful soil and plant sampling will reinforce the accuracy and precision needed in studies designed to quantify soil fertility in terms of soil composition. These studies often depend on the establishment of relationships between crop yield parameters and soil physio-chemical and biological properties or analyses. For example, regression studies may be done with parameters representing yield response to fertilizers as dependent variables and soil analyses as independent variables, as in soil test calibration studies (e.g. Osborne, 1974). When the yield parameters are estimated by field experiments, the laboratory analyses are expected to represent the sites on which the experiments are done and the soil and plant sampling should be sufficiently intensive to capture the variability within the site.

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CHAPTER 4

SAMPLE PREPARATION AND LABELLING

Each sample should be clearly labelled in order to reduce mis-assigned values to samples. Remember that as little as one pair of confused samples can result in an experiments inability to differentiate between key treatments and in turn lead scientists to conclude that treatment effects do not exist when in fact they do. If large numbers of samples are being collected, a valuable safety precaution is to label each sample twice, one on the outside of the sample container, the other inside. Labelling codes should be developed that are compact and easily understandable and those codes must be entered into data notebooks prior to the collection of samples. When samples are received in the laboratory from the field, they are logged-in by a responsible person, it is common practice to find laboratories whereby, the exercise of sample reception is done by anyone. This more often than not results to a confused state of affair.

Sample log-in is a very important step for the purpose of retracing a sample that may have been lost. A manual log-in is done in internal laboratory registers immediately upon reception of the samples, however other laboratory may use computer log-in, but in either way should carry detailed information. Various systems are used for this identification, using numbers or alphanumeric for example, a letter may be used to designate the matrix such `s` for soil and `p` for plant `m` for manure etc followed by a consecutive assigned numbers. For example, if the effects of three N levels are being investigated in a thrice-replicated experimental design at three different farms and these samples are being collected at 4 intervals. It is more advantageous to label the first sample Site 1; Treatment 1, Replicate 1; Time 1 (or S1Tr1R1T1) rather than MacDonald's Farm poultry manure 27-3-93 number 1. The important point here is, no two samples can have the same identity. The log-in information should also contain the name of the client and the type of the analyses requested. More detailed records of sample collection can be maintained by the researcher and field technicians that include the labelling code, sampling depth, calendar date, etc. Analytical laboratories that fulfil a service function are well advised to maintain sample information sheets. These samples are most often numbered sequentially during a given year. An example information sheet is presented in Table 4.1.

Soil samples are best collected in plastic bags or tubes, whereas plant materials should be collected in paper bags to reduce decomposition prior to preparation for analysis. Examine plant samples for contamination from pesticides, fertilisers, dust or mud. A brief rinse with deionised water may be required. All soil samples should be air dried as soon as possible, unless the test requires a fresh, field moist sample, as is the case when soil biological activities are being measured.

Sample Handling and Choice of Analyses. An analytical procedure generally consists of three steps: Sampling, pre-treatment and determination. In the past, the attention of analytical chemists was paid mainly to the determination step, in particular for developing more sensitive and more selective methods. At present there are indeed such methods, but this implies that it is even more important to apply a suitable pre-treatment so that valid analytical results will be obtained. For example, the total content of an element in a soil sample can be determined after complete destruction of the sample, but more delicate pre-treatment (e.g., extraction) is required in order to distinguish the various types of compounds which include that particular element (speciation). In general, the untreated sample gives a gross signal that may deviate largely from the net analytical signal; therefore the pre-treatment is often called clean-up.

The pre-treatment of a sample before the actual determination can take place is a necessary evil, since most matrices (soil, plant tissue, food, feed, etc.) do not allow a direct measurement of the test

Table 4.1. Sample information sheet for a service laboratory.

Sample number _____ date _____

Name of client _____

Address of client _____

Type of sample _____ plant _____ soil

Incoming sample label	_____	content	cost
Analyses to be conducted	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

For plant samples: crop _____ date planted _____

date sampled _____ growth stage _____

plant part _____ crop condition _____

Additional comments _____

_____

For soil samples: cropping history _____

rainfall past 30 days [normal / below average / above average]

fertilisers applied: type _____

amount _____

date _____

Additional comments _____

_____

location and sketch map of site (draw map on opposite side of page) _____

_____

substrate. The causes can be classified into three categories: 1) the substrate is inaccessible, 2) the substrate is in unsuitable form or 3) the signal is interfered by contaminants.

The substrate is inaccessible when it occurs inside plant cells, or "trapped" between crystal layers of a clay mineral. The test substrate may be in unsuitable form for the intended method of determination when for instance nitrogen is to be measured as ammonia while it is organically bound. The natural matrix of a sample (the whole of concomitant compounds) often causes heavy interference by affecting the analyte signal; for example, the measurement of low concentration of nitrate in plant tissue extracts by means of an ion-selective electrode yields too high results because organic anions contribute to the signal. Destruction or extraction is therefore, a process by which, the analyte is released; it may at the same time react (mostly by oxidation) to a form suitable for measurement. This process is also called opening out, mineralisation, ashing, or digestion. In the case of extraction, the matrix is not broken down; on the contrary, the analyte is removed from the matrix as of selective as possible.

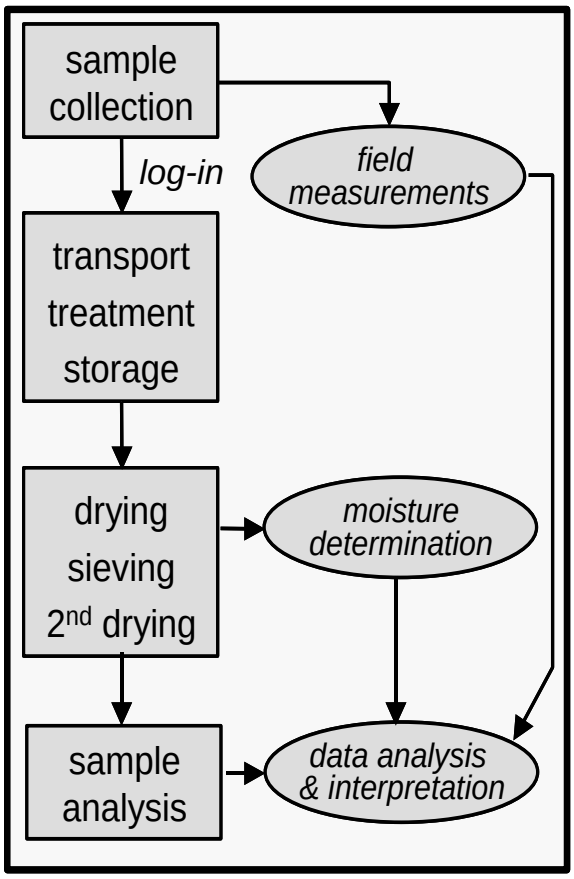

Figure 4.1. Flow diagram of sample collection, handling, storage and analysis.

Handling Procedure of Soil Samples

1. Air-dry the soil by placing it in a shallow tray in a well-ventilated area or forced air oven at 40°C. Break up any clay clods. When the soil is dusty it is dry enough.
2. Crush the soil lumps so that the gravel, roots and large organic residues become separated. Avoid smashing any soft gravel.
3. Sieve the soil through a 2 mm sieve, and gently rub the crumbs through the mesh leaving the gravels and roots etc. in the sieve. Pick out the roots and save if required.
4. If the proportion of soil retained by the 2-mm sieve is to be quantified, then both the dry weight of soil retained by and passing through the sieve must be measured. This should be done if gravel-sized particles constitute > 5% of the sample.
5. Retain a representative sample of approximately 250-g by coning and quartering.
6. Soils are further ground in a mortar in order to pass through a 60 mesh screen for total N, organic C and P analysis.

Drying. For most soil and plant analyses, drying is a step that cannot be overlooked. Usually soils are air-dried or oven dried in forced air ventilation at 40^{o}C while plant samples are oven-dried between 60-70^{o}C. The samples should be dried as quickly as possible to overcome the eventual decomposition processes, which may change the original chemical composition. Thickness of the soil layer while drying should be limited to 1.5-2cm. Studies have shown a marked chemical composition being influenced by drying at different temperatures

Sieving and Milling. When samples are logged in the laboratory register, and dried as above, larger particles of the soils, which are loosely bound to each other, are crushed in pestle and mortar, a small force will make them fine. This is then passed through 2mm sieve accepted standard sieve mesh, for all the analyses that require sample weight >2g. All the analyses that require sample

weight <2 g weight, further finer sieving is necessary, about 250 μm is sufficient. After the drying the sample must be milled to the <2 mm using an electric mill. It is important to hammer them so as pass through the required mesh size. Pestle and mortar or an electronic milling machine can do this. The finer the sample, the more homogeneous it is.

Water and Acid Extractions. Water is used for the extraction of some anions like nitrate, nitrite, chloride and sulphate from plant material. After shaking a suspension with a liquid/solid (=l/s) ratio of 50 for half an hour at room temperature, the total contents of these anions can be determined. Since the extract is a very good culture medium for mould growth, the measurements should be taken on the same day. Silicates and borates can be extracted from plant material by a mixture of hydrochloric acid and hyfluoric acid. As the extract is turbid and coloured brown, it cannot be analysed by spectrophotometer; the recommended method is ICP emission spectrometry (after filtration to prevent clogging of nebuliser). The use of Hf requires consequent application of polythene or polycarbonate plastic ware in order to avoid pollution of the solutions by Si or D from glass.

Dry Ashing. Plant material can be digested conveniently by simply heating the dried and ground substance in a muffle furnace to such a high temperature that all carbon-containing compounds are volatilized or oxidised. This method is called dry ashing, since no liquid is added and a (white) ash is produced that consists solely of inorganic salts. After cooling down, the sample ash is taken up in acid for determination of elements. Dry-ashing is simple to carry out, but there are some pitfalls:

- **Reactions leading to combustion**. The temperature of the sample may become 150^{o}C higher than that of the furnace. Eventually the sample may spontaneously set on fire, which may cause self-combustion. When the furnace with the samples is heated up, the temperature of a sample causes loss of analyte. Therefore, the plant samples should first be charred carefully on a hot plate.
- **Choice of temperature**. If the final temperature remains too low, the sample will not be ashed completely; this is evident from the grey or black colour of the residue. At too high temperatures, however, the analyte may volatilise, this process cannot be observed directly. The best temperature range has turned out to be 450-550^{o}C. The actual selection of the ashing temperature depends also on the composition of the matrix, many metal halides, for example, are rather volatile, so that e.g. heavy metal ions can be lost as their chlorides when the temperature is chosen too high.
- **Contamination**. In some dry-ashing procedures, addition of a reagent is necessary for better oxidation or to prevent volatilisation. This reagent should be very pure avoiding contamination.
- **Inclusion**. A black or grey ash implies the presence of carbon, which may include analyte. After taking up the ash in acid and filtration, the process should then be repeated at higher temperature (but max. 550^{o}C) with the ash that remains on the filter.
- **Fixation**. When using porcelain crucibles at high temperatures, metal ions(e.g., Al, Cu, Fe, Pb, Zn, Al) may be bound to the silicates of the plant, and should be released by fuming with HF. When platinum crucibles are used, some metals (e.g., Ag, Cu, Hg, Pb, Sn) may be lost for analysis by formation of alloys.

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CHAPTER 5

THE BASICS OF SPECTROPHOTOMETRY

Instruments are devices that measure the chemical or physical properties of substrate. Equipment is a device that does not measure a property, but rather influences a substrate. Examples of equipment are sieves, ovens, hot plates and centrifuges. Both equipment and instruments are necessary in virtually every laboratory analytical procedure. Many instruments rely upon the phenomenon of spectrophotometry, and this chapter is intended to provide an overview of the principles and devices utilized with the laboratory.

The chemical characteristics of matter give rise to analysis of its nutrient components. Matter is composed of atoms and the atoms contain negatively-charged electrons, which occupy orbitals around the nucleus (protons and neutrons). Different orbitals are associated with specific energy levels. When electrons are subject to external energy, they become excited and occupy higher energy orbitals but at an unstable state. When electrons return to their previous state, energy is released as radiation. Bohr`s theory of atomic spectra was later modified by Sommfield to explain the quantum theory, thus when an electron jumped from higher energy level to a lower energy state a quantum of energy equal to *hv* is emitted where $hv = En - Em = \Delta E$ with $E_n > E_m$ being the energies of two quantised energy levels. These characteristics in energy levels are element specific and are therefore used for the analysis for different nutrient elements.

In atomic spectroscopy, two modes of radiation behavior are possible, emission or absorption. With emission, the atom receives heat energy and becomes excited momentarily but loses the extra energy in form of light (Figure 5.1). With absorption, energy in form of light excites the substrate to measurably higher levels. Absorption and emission have been widely employed in field of quantitative and qualitative elemental analyses. Atomic absorption, ultraviolet and visible spectrophotometers are three common laboratory instruments that employ these principles.

Quantification is achieved through the use of Beer's Law that states $\boldsymbol{A = a^* b^* c}$ where **A**= absorbance, ***a*** = absorption coefficient, ***b*** = path length and **c** = the concentration of the substrate that is analyzed. Both ***a*** and ***b*** are constant for a given element and set instrument conditions, therefore the equation may be simplified to $\boldsymbol{A = k^* c}$. Thus a direct relationship exists between absorbance measured by the instrument and the amount of substrate responsible for that property. Spectrometric equipment consists of five parts: a light source, a monochromator, a sample cell, a detector and a read-out system. There are large differences in price and performance of different commercially-available instruments, but all rely upon the basic principles described above.

There are two basic spectrometer designs: single-beam and double-beam operations. Single-beam instruments are particularly suitable for quantitative measurements at fixed wavelengths (5.2). Double-beam instruments are expensive, because they contain more optical components. But they have the advantage of allowing to scan an absorption spectrum. In a double-beam system there are two cuvettes, one with sample solution and one with reference solution (preferably the solvent blank). The incident light beam falls alternatingly on either cuvettes by means of chopper thus the ratio I/I_o is measured many times per second; in other words there is nearly continuous measurement of transmittance. As a consequence, all kinds of variations like lamp intensity and detector response do not influence the sample signal, since I_o is measured at virtually the same conditions.

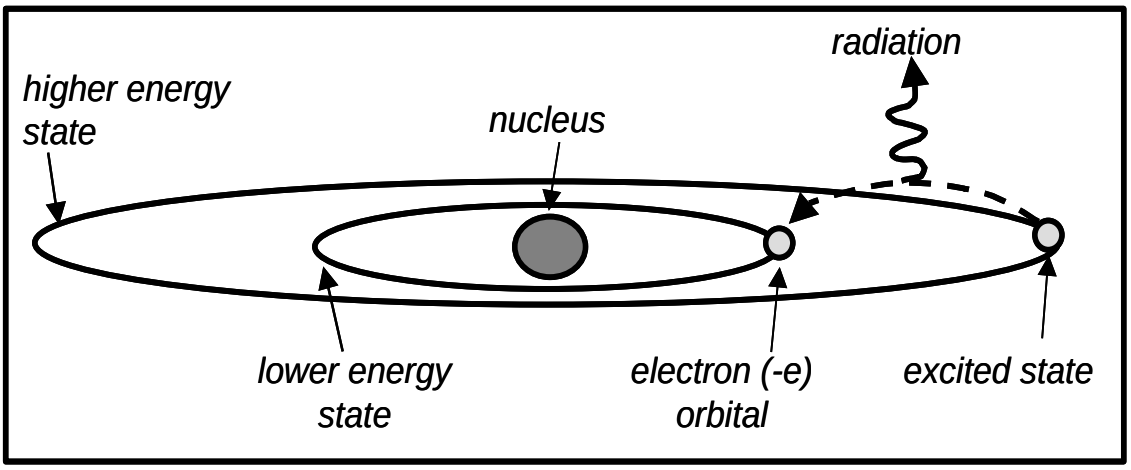

Figure 5.1. Emission results when radiation is released as an electron falls from a higher to a lower energy state.

Light Source. The light source can be a simple tungsten lamp suitable for the visible part of the spectrum. This lamp contains a 3 mm wide strip of tungsten; heated by an electric current to about 2500 ˚C. Its radiation has a maximum intensity in the near infrared but decreases rapidly in the UV range for wavelengths shorter than 300 nm this lamp cannot be used. Another possibility is the hydrogen discharge lamp, filled with H_2 gas at pressure of about 1 kpa (0.01 atm) at wavelengths shorter than 350 nm this lamp produces a continuous spectrum of medium intensity by excitation of H_2 molecules. The intensity increases when hydrogen is replaced by deuterium. The mercury lamp produces a line for scanning an absorption spectrum, but it is suitable for quantitative measurement s of absorption and fluorescence at fixed wavelengths of one of the mercury lines in the UV range. In all cases the power supply voltage and the current are stabilized in order to have a constant I_o.

Monochromator. As already mentioned the linear relationship between absorbency and concentration holds only if monochromatic light is used. To obtain such light, a source with continuous spectrum is used, part of which is isolated by a filter or a monochromator. The quality of this light is determined by the resolution power of the filter or the monochromator and is expressed as its band pass (bandwidth). The band pass is normally 50 to 100 nm for colored glass, about 20 nm for a good glass filter combination and about 10 nm for an interference filter. Monochromator is the name for a module that consists in general of an entrance slit, a collimator (lens or mirror), a grating or prism, again a collimator and an exit slit. Older spectrometers are often equipped with prism, which may have wavelengths down to 1nm. However, the material's natural (CAF2) is very expensive and the band pass aren't constant. Actually, the wavelength decreases with increasing λ; it is compensated by varying the exit slit width, but this makes such apparatus to have a very complex mechanical design. At present nearly all spectrometers are equipped with holographic reflection gratings.

Formerly, gratings were made by mechanical rulings of grooves (600-6000 per mm) in glass surface, which is very difficult and thus very costly. Use was made of therefore of replicas from original gratings nowadays gratings are prepared conveniently by projecting a laser interference pattern on a light sensitive surface. After processing this so-called holographic grating is ready for use no replicas are needed. Holographic grating are now applied almost exclusively, because they are cheap have a constant band pass of down to 0.2 nm and provide a very low lever of stray light.

Sample Cell. The solution to be contained in a rectangular shaped sample cell or cuvette, made of glass (for work involving the visible spectrum) or quartz (for UV spectrophotometry). Both the incident and the emergent surfaces should be perfectly flat parallel to each other and free from

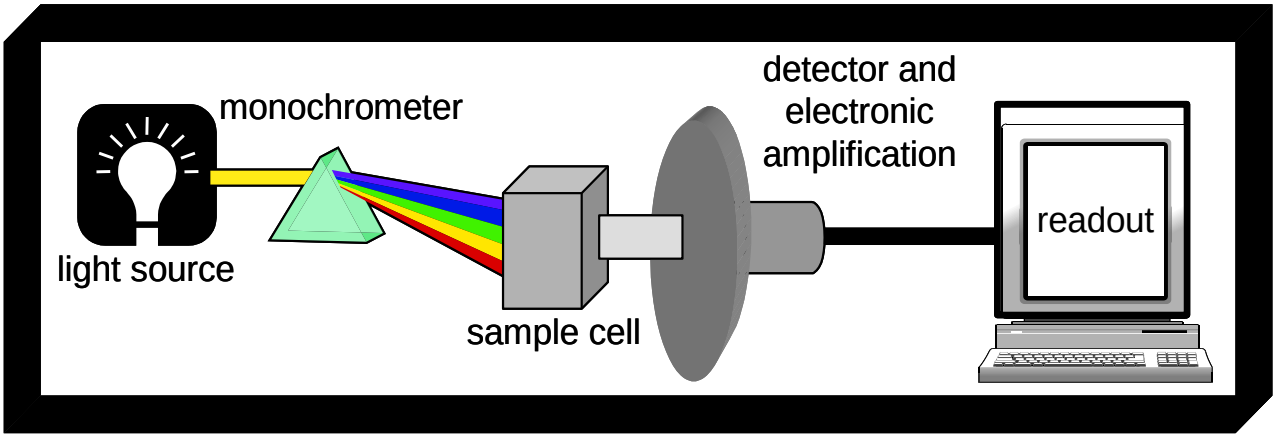

Figure 5.2. A simplified diagram of single-beam spectrometer where energy emissions or absoption from a sample is captured by a detector and directed to a readout device.

scratches. The path length is almost 10 mm but a wide variety of lengths and shapes can be purchased. In single beam arrangement, the sample and blank are measured alternately. In both systems the cuvettes must be matched. In many automatic instruments, one (flow through) cuvette is used alternatingly and the cell is always placed behind the monochromator to avoid decomposition of the sample.

Detector. The detector may vary from a simple selenium photocell that can be connected directly to a galvanometer to phototubes or photo-multiplier tubes with an electronic amplification and measuring circuit. Nearly all-modern spectrometers are equipped with photo-multiplier tubes. In a simple phototube, photons strike the cathode coated with photosensitive material and release electrons, which are collected on the anode, in this way light energy is transformed to electric energy directly. Photo-multiplier tubes have similar photo-cathode, but the released electrons are accelerated by a potential difference of e.g. 100v. The accelerated electrons release a number of secondary electrons on the first dynode. On the next dynodes the process repeats itself. In this way an amplification of 10^6 can be achieved easily.

Read-Out System. In early spectrometers, the electric signal coming from the detector used to be read out on the galvanometer only. Later on the signal could be registered by means of chart recorders (analogous) or printer (digitally). Nowadays more and more instruments use microprocessors to present the result on digital display, to store them on tape on to disc and to do any further computation.

Scanning an Absorption Spectrum. When dealing with an unknown substance, the variation of its absorption with wavelength $\Delta\lambda$s must be known in order to choose a suitable wavelength $\Delta\lambda$s for quantitative determination. The relation of absorption coefficient versus wavelength is called the absorption spectrum of a substance. Scanning such a spectrum is done by slowly turning the monochromatic so that light with gradually changing wavelength is passed through the unknown substance, while the absorbance is measured. So-called scanning spectrometers are able to scan and record a spectrum completely automatically. The latest instrument has a microprocessor which enables them to present the spectrum on a display to calculate and present the spectrum the first, second derivative, to enable absorption minima and maxima. A diode array detection: a large number (>300) of tiny photosensitive diodes arranged linearly on a 12mm chip. Each diode views a spectrum band of only 2mm width so that a total range of more than 600 nm is covered (from 190 to >800 nm). This kind does require turning the monochromator

CHAPTER 6

SOIL PARTICLE SIZE ANALYSIS BY THE BOUYOUCOS OR HYDROMETER METHOD

Principle. The particle size analysis of a soil estimates the percentage sand, silt and clay contents of the soil and is often reported as percentage by weight of oven-dry and organic matter-free soil. The analyses are usually performed on air-dry soil. Based on the proportions of different particle sizes, a soil textural category may be assigned to the sample. The first stage in a particle size analysis is the dispersion of the soil into the individual particles. These are the sand (2.00 - 0.05 mm), silt (0.05 - 0.002 mm) and clay (< 0.002 mm) fractions. Individual soil particles are often bound into aggregates hence the requirement for dispersion. The hydrometer method of silt and clay measurement relies in the effects of particle size on the differential settling velocities within a water column. The settling velocity is also a function of liquid temperature, viscosity and specific gravity of the falling particle. Theoretically the particles are assumed to be spherical and to have a specific gravity of 2.65. If all other factors are constant then the settling velocity is proportional to the square of the radius of the particle (Stoke's law). In practice, therefore, we must know and make correction for the temperature of the liquid. Greater temperatures result in reduced viscosity due to liquid expansion and a more rapid descent of falling particles.

Reagents and Apparatus

1. Calgon (sodium hexametaphosphate) solution, 10%. Dissolve 100 g of calgon in 1 litre of distilled water. Note: this solution should not be kept over one month, when too old it loses its dispersing efficiency.
2. Amyl Alcohol
3. Hydrometer with Bouyoucos scale in gram per litre
4. Soil dispersing stirrer. A high speed electric stirrer with a cup receptacle.
5. Reciprocating shaker.

Procedure

1. Weigh out 50 g of air-dry < 2 mm soil (100 g in case of very sandy soil) into a 400 ml beaker.
2. Saturate the soil with distilled water and add 10 ml of 10% Calgon solution. Allow to stand for 10 minutes.
3. Transfer the suspension to the dispersing cup and make to the mark in the cup with distilled water.
4. Mix the suspension for 2 minutes with an electric high speed stirrer. Use ordinary bottles if cup is not available. Shake the suspension overnight on reciprocating shaker if stirrer is not available.
5. Transfer the suspension into a graduated cylinder and rinse remaining soil into the cylinder with distilled water. Insert the hydrometer into the suspension and add water to 1130 ml, then remove the hydrometer.
6. Cover the cylinder with a tight-fitting rubber bung and mix the suspension by inverting the cylinder carefully ten (10) times. Note the time.
7. Quickly add 2 - 3 drops of amyl alcohol to the soil suspension in order to remove froth and after 20 seconds place the hydrometer gently into the column.
8. At 40 seconds, take a hydrometer reading and measure the temperature of the suspension.
9. Repeat step 6 (mixing of the soil suspension 10 times) and allow the cylinder to stand undisturbed for 2 hours. After two hours, take both hydrometer and temperature readings.
10. Make the necessary temperature corrections (Table 6.1). Temperature affects the hydrometer readings and, because the hydrometer has been calibrated at 68°F (20°C), either correction factors must be applied or the determination conducted in a temperature controlled room kept at the correct temperature.

Table 6.1. Temperature correction for hydrometer readings of soil texture.

Temperature ^{o}C	Hydrometer correction g per litre
15	- 2.0
16	- 1.5
17	- 1.0
18	- 1.0
19	- 0.5
20	Nil
21	+ 0.5
22	+ 1.0
23	+ 1.0
24	+ 1.5
25	+ 2.0

Calculations. To calculate sand as %: after 40 seconds, the sand has settled and the hydrometer reading reflects the grams of silt + clay in l litre of the suspension. To calculate the amount sand present in 1 litre of the suspension, subtract this value from the original sample weight.

Sample Calculation. If the hydrometer reading after 40 seconds corrected for temperature is 18.0 g per litre, then silt + clay weigh 18.0 g in the 1 litre soil suspension. Therefore, the sand weighs 50.0 - 18.0 = 32.0 g in the 1 litre suspension (of the original 50.0 g of air-dry soil sample). The percentage sand is calculated by dividing the sand content (32 g) by the total (50 g) and multiplying by 100 as follows:

$$\text{sand \%} = (32.0 / 50.0) \times 100 = 64\%$$

Clay. After 2 hours, the silt has settled. The hydrometer reading now reflects the clay content of the original suspension. For example, if hydrometer reading after temperature correction is 4.7 g/litre, then the percentage of clay in soil is:

$$\text{clay \%} = (4.7 / 50) \times 100 = 9.4\%$$

Silt. The silt content is calculated by subtracting the sum of the clay and sand contents from 100% or:

$$\text{silt \%} = 100 - (9.4\% \text{ clay} + 64\% \text{ sand}) = 26.6\%$$

The assumption made here is that the organic matter is negligible. However, if soil is found to be high, treat the soil with hydrogen peroxide until the frothing reaction subsides. An alternatively, determine the organic matter content and subtract it from 100 before assigning it in the formulae.

Soil texture. Once the sand, silt and clay distribution is measured, the soil may be assigned to a

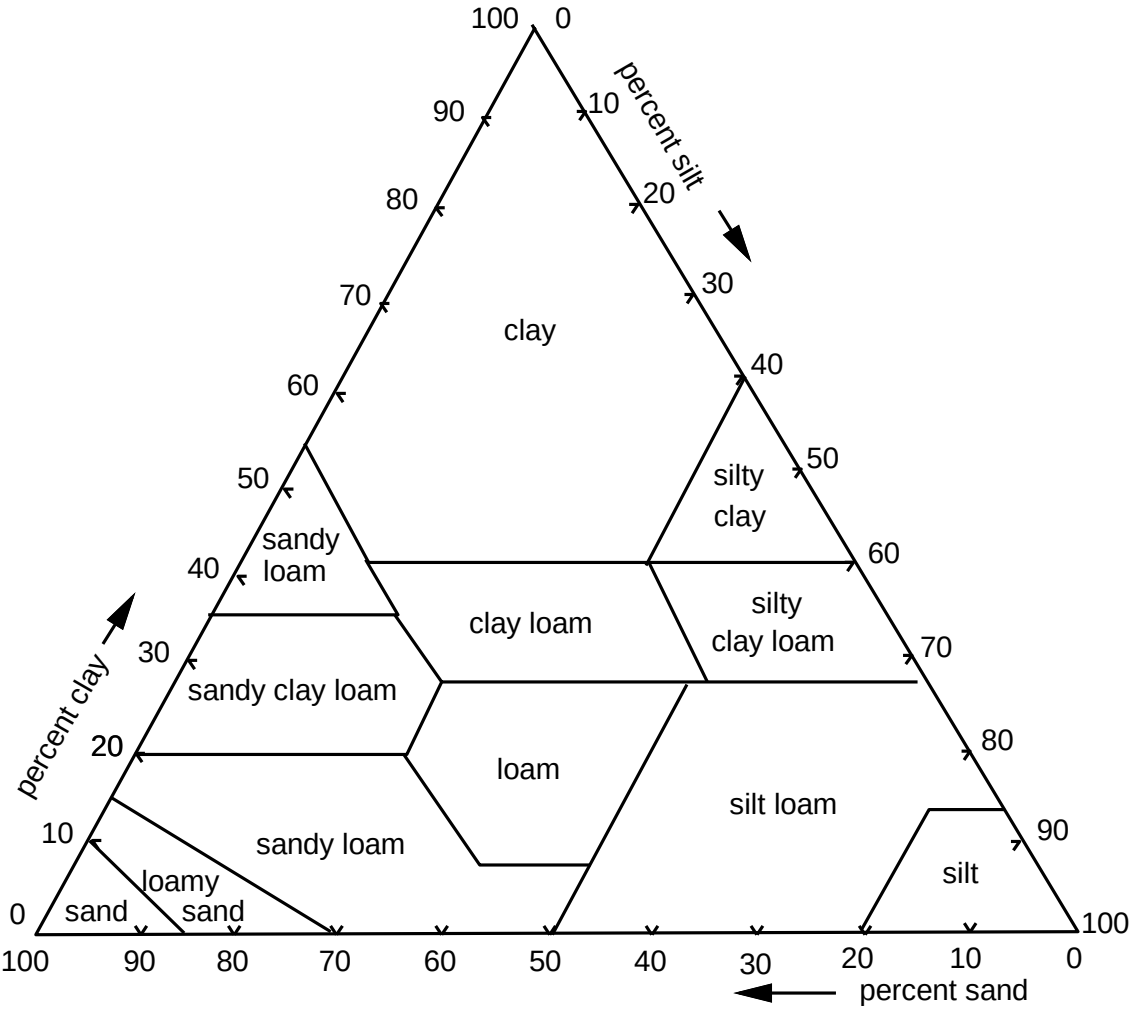

Figure 6.1. Soils may be assigned to textural classes based on particle size distribution using the soil textural triangle.

texture class based on the soil textural triangle (Figure 6.1). Within the textural triangle are various soil textures which depend on the relative proportions of soil particles. Users simply obtain the appropriate texture based on the particle size distribution. In the example above (64% sand, 27% silt and 9% clay), the corresponding soil texture is a sandy loam.

Procedural notes

1. Cylinders for particle size analysis are calibrated depending upon the volume of the hydrometer in use. At NARC Muguga, the calibration is 1130 ml, indicating the final volume of the soil suspension with the hydrometer inserted.
2. Many laboratories have developed their own temperature conversion tables depending on the exact procedure and working conditions.

References

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CHAPTER 7

SOIL BULK DENSITY AND WATER HOLDING CAPACITY

Principle. The bulk density of soil is the mass per unit volume expressed as g cm^{-3}. Once the bulk density is known, measurements of soil mass, volume or percentages can be expressed interchangeably or expressed in absolute terms (e.g. from g kg^{-1} soil to kg ha^{-1}). For example, a surface soil with a bulk density of 1.5 g cm^{-3}, a depth of 10 cm and containing 3% total organic carbon contains 150 kg soil m^{-2} (100 cm x 100 cm x 10 cm x 1.5 g cm^{-3}) and 4500 g total organic C m^{-2} This organic carbon is equivalent to 45,000 kg C or 45 t ha^{-1}. Thus, expressing soil data on an area basis requires that the soil bulk density be known. The water holding capacity of a soil is expressed as the moisture content of a freely drained, oven dried soil. Once a soil is freely drained, the remaining moisture is held at between -0.1 and -0.3 bar. The lower limit of plant available water is -15 bar the difference between field capacity and the lower limit is the plant available water content. The procedures described in this chapter are critical in the conversion of laboratory data to actual field conditions and is largely based upon those described by Anderson and Ingram (1993).

Bulk Density of Non-stony Soils

Procedure

1. Remove 1-2 cm of surface soil from the level area where samples are to be measured.
2. Insert a 5 cm diameter thin-sheet metal tube of known weight (W1) and volume (V) 5 cm into the soil surface.
3. Excavate the soil from around the tube and cut the soil beneath the tube bottom.
4. Remove excess soil from the tube ends using a knife.
5. Dry at 105°C for 2 days, and weigh (W2).

Calculation Bulk density (g cm^{-3}) = (W2 g - W1 g)/ V cm^3

Bulk Density of Stony Soils

Procedure

1. Excavate an intact clod of soil.
2. Air-dry the clod, tie a thin thread around it and weigh (W_s).
3. Dip it briefly in melted paraffin wax (60°C) in order to waterproof the clod.
4. Weigh the coated clod (W_{sp}) and calculate the weight of the paraffin coating (W_p)
5. Where ($W_p = W_{sp} - W_s$).
6. Suspend the clod from the balance arm and submerge it completely in a beaker of water. Record the weight (W_{spw}). If it leaks air, discard the clod.
7. Break the clod open, take a subsample of the soil and determine the moisture content.
8. Correct W_s to its oven dry mass:

$$W_{dry} = \frac{W_s \text{ x weight of subsample after drying}}{\text{weight of subsample before drying}}$$

Calculation.

$$\text{Bulk density (g cm}^{-3}) = (W_{dry}\ g)/[((W_{sp}\ g - W_{spw}\ g)/D_w\ cm^3)) - (W_p\ g/D_p\ cm^3)]$$

where D_w = density of water at temperature of determination (1.0) and D_p = density of paraffin wax (approximately 0.9).

Bulk Density by the Soil Infill Method

Procedure. If soil conditions are such that the above method is impractical or the soil shrinks and swells, the infill method may be employed: Dig a hole approximately 10 cm x 10 cm x 10 cm. Dry all the soil recovered at 105°C for 24 hr and weigh (W). Fill the hole with dry coarse sand from a known volume of sand. Make sure that the sand surface is level with the adjacent soil surface. Record the volume of sand remaining and hence calculate the volume used to fill the hole (V).

Calculation. Bulk density ($g\ cm^{-3}$) = W g/V cm^3 or

Bulk density ($g\ cm^{-3}$) = Dry Weight of removed soil (g) / Volume of replacement sand (cm^3)

Bulk Density for Shrink-Swell Soils (Vertisols)

Approach and Calculation. In shrink-swell soils the wet bulk density and the dry bulk density differ. It is difficult to measure when the soil is either very wet or very dry. The minimum bulk density, which occurs when the soil is fully wet and expanded, is estimated by assuming that the particle density is 2.65 $g\ cm^{-3}$ and air content when field saturated is 0.03$cm^3\ cm^{-3}$ (Shaw and Yule, 1978). Hence:

$$\text{Bulk Density } (g\ cm^{-3}) = 1/(W_{max} + 0.4046)$$

where W_{max} is the maximum gravimetric water content of the horizon in question, the field saturated water content.

Field Water Holding Capacity

Procedure. Field capacity is defined as the maximum amount of water the freely drained soil can hold and is estimated after a saturated soil has been allowed to drain without allowing its moisture stores to be depleted by evaporation. The method is as follows:

1. Build an earth bund around a 1 m x 1 m area, and fill with water.
2. Refill with water as necessary so that approximately 50 cm of water has soaked into the soil.
3. Cover the area with a plastic sheet in order to prevent evaporation and leave for 2 days.
4. Bulk 5 replicated 0-10 cm soil samples from near the centre of the area.
5. Put about 250 g of the wet soil in a moisture can of known weight (W1), weigh (W2), then dry at 105°C for 48 hr. Weigh the dry soil again with the moisture can (W3).

Calculation $$\text{Soil moisture at field capacity (\%)} = \frac{(W2 - W3) \times 100}{(W3 - W1)}$$

The Lower Limit of Plant Available Water

Procedure. This value is sometimes known as the wilting point and is often equated to the soil water content at -15 bar (or -1.5 MPa) water potential. This value is obtained as follows:

1. Distribute rubber sample rings or metal rings with cheesecloth fastened to one end with a rubber band around a pre-soaked -15 bar ceramic plate.
2. Fill each ring with soil. Do not compress or pack the soil into the ring. Prepare triplicate samples.
3. Place the plate in a large tray and slowly add tap water until the water is about half way to the top of the sample rings. Soak the samples overnight.
4. Seal the outflow tube on the ceramic plate with a clamp. Carefully drain excess fluid out of the tray. A syringe or siphon works well.

5. Place the plate (with samples) in the pressure chamber. Connect the outflow tube of the ceramic plate to the fitting on the inside of the chamber. Connect another tube to the fitting on the outside of the chamber, and place the free end of the tube in a beaker of water. Unclamp the outflow tube so that water may flow freely from the ceramic plate to the beaker on the outside of the chamber.
6. Place a damp cloth over the samples in the chamber to maintain high humidity while the samples are equilibrating. Close the chamber, tighten the wing nuts, and slowly apply air pressure to the chamber until 15 bar is reached.
7. Allow the samples to equilibrate for 2 to 4 days. The longer time is for soils with high clay contents. Before releasing air pressure, clamp the outflow tube so that water may not re-enter the ceramic plate. Release pressure slowly. Open the chamber and remove the samples. Determine the water content.

Calculation

The lower limit of plant available water (%) = [(W2 - W3) / (W3 - W1)] / 100

where W1 = weight of container (g), W2 = weight of container + wet soil (g) and W3 = weight of container + oven dry soil.

Plant-Available Water Holding Capacity

Approach and Calculation. The amount of water which a given soil horizon can store for plant use is estimated from the difference between the field capacity and lower limit of plant available water for that horizon. It is expressed as an equivalent depth of water (mm), and is calculated as follows:

$$PAWC = (\text{field capacity} - \text{lower limit}) \times BD_{soil} \times Z$$

where BD_{soil} is the bulk density of the horizon and Z is the thickness of the horizon in mm. The total PAWC is the sum of the PAWC of all the horizons down to the effective rooting depth.

Moisture Determination for Dry Weight Data Correction

Procedure

1. Weigh about 1 ± 0.001 g of prepared air-dry material into a dry container of known weight (W1). Record the weight (W2). Dry at 105°C for 2 hr, allow to cool in a desiccator and weigh (W3).
2. Correct all data to a dry weight basis by multiplying with (100 / dry material in %).

Calculation

Dry material (%) = [(W3 - W1) x 100] / (W2 - W1)

References

Anderson, J.M. and Ingram, J.S.I. (1993). *Tropical Soil Biology and Fertility: A Handbook of Methods*. CAB International, Wallingford, UK. pp. 95-99.

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CHAPTER 8

SOIL pH AND ELECTROCONDUCTIVITY

Principle. Measurement of pH is expressed as the inverse log of the hydrogen ion concentration. The pH of the soil solution controls the form and solubility of many plant nutrients. Soil pH is measured on 2.5:1 soil water suspension. The electroconductivity measurement identifies soils which are potentially saline. The electroconductivity of the saturated paste extract is measured to determine the level of salinity.

General Procedure for Soil pH (2.5:1 H_2O)

1. Add 50 ml deionised water to 20 ± 0.1 g soil.
2. Stir the mixture for 10 minutes, allow standing for 30 min and stirring again for 2 min.
3. Measure the pH of the soil suspension.
4. Allow to settle for 1 hr then measure the conductivity of the supernatant liquid. For samples with an EC > 1.0 mS cm^{-1} consider saturated paste extract analysis (procedure follows).

Saturated Paste Extract Conductivity

Apparatus. Conductivity meter and pH meter

Reagents and standards

1. Dissolve 0.7456 g of KCl in 1000 ml water: 1.412 mS/cm at 25°C.
2. Dissolve 7.456 g of KCl in 1000 ml water: 12.900 mS/cm at 25°C.
3. Buffer solutions pH 4, 7 and 9.2.

Procedure

1. Weigh about 300 ± 25 g soil into a plastic container.
2. Add water to the soil with stirring until it is nearly saturated.
3. Allow the mixture to stand covered for several hours to permit the soil to imbibe the water, and then add more water to achieve a uniformly saturated soil-water paste. At this point the soil paste glistens as it reflects light, flows slightly when the container is tipped, slides freely and cleanly off a spatula, and consolidates easily by tapping or jarring the container after a trench is formed in the paste with the slide of a spatula.
4. After mixing, allow the sample to stand (preferably overnight, but at least 4 hr), and then recheck the criteria for saturation. Free water should not collect on the soil surface, nor should the paste stiffen markedly or lose its glisten. If the paste is too wet, add additional dry soil to the paste mixture.
5. Transfer to a Buchner filter funnel fitted with highly retentive filter paper. Apply vacuum, and collect the filtrate. If the initial filtrate is turbid, refilter.
6. Measure the conductivity of filtrate against that of the standards.
7. To categorise soils as saline or non-saline, see Rhodes et al. (1982).

Reference

Rhoades, J.D. (1982). *In Methods of Soil Analysis, Part 2*. Second Edition (A.L. Page, R.H. Miller and D.R. Keeney, Eds.). American Society of Agronomy. Madison, USA.

CHAPTER 9

TOTAL NITROGEN AND PHOSPHORUS IN PLANTS AND SOILS

Principle. The content of total nitrogen and phosphorus is measured in a digest obtained by treating soil and plant sample with hydrogen peroxide+sulpuric acid+selenium and salicylic acid. The principle takes into account the possible loss of nitrates by coupling them with salicylic acid in an acid media to form 3-nitrosalicylic and or 4-nitrosalicylic. The compounds are reduced to their corresponding amino acid forms by the soil organic matter he Analysis of total nutrients requires complete oxidation of organic matter. The hydrogen peroxide oxidises the organic matter while the selenium compound acts as catalyst for the process and the H_2SO_4 completes the digestion at elevated temperatures.

The main advantages of this method are that single digestion is required (for either soil or plant material) to bring nearly all nutrients into solution; no volatilisation of metals, N and P takes place and the method is simple and rapid. It is to be noted that various wet ashing procedures have been proposed world-wide, depending mainly on the equipment, sample targets and the cadre of personnel. However, the procedure presented in this chapter is fast, accurate and reproducible.

Reagents (Analytical reagent grade ('AR') chemicals are highly recommended)

1. Selenium powder, Se
2. Salicylic acid
3. Hydrogen peroxide, 30%, H_2O_2 (or 100 vols.)
4. Sulphuric acid H_2SO_4, concentrated
5. Sulphuric acid and selenium powder mixture: dissolve 3.5-g of selenium (1) in the 1 litre of sulphuric acid (4). Heating to about 300^0C while covering the beaker with a watch glass. The author's laboratory has proved that butane gas flame does very well because of the ability to heat uniformly all the sides of the beaker. The originally blackish colour of the selenium suspension turns via green blue to clear light yellow.
6. Digestive mixture: dissolve 3.2-g salicylic acid (2) in 100-ml of sulphuric acid-selenium mixture (5). This mixture should not be stored for more than 48 hours.

Remarks. The moisture of the plant sample must be kept very low by drying at 70 ^{o}C before weighing, this is found to have a moisture content between 1-2 %. Hydrogen peroxide should be analytical grade to reduce any interfering compounds e.g. phosphate.

If the moisture content is not low enough it will have sudden reaction with the concentrated sulphuric acid which raises the temperature causing loss of nitrates.

Procedure Using a Block Digester

1. Weigh 0.3 ± 0.001 g of oven dried (70°C), ground plant tissue or soil (< 0.25 mm, 60 mesh) into a labelled, dry and clean digestion tube.
2. Add 2.5-ml digestion mixture to each tube and the reagent blanks for each batch of samples.
3. Digest at 110°C for 1 hour. Remove, cool and add three successive 1-ml portions of hydrogen peroxide.
4. Raise the temperature to 330^OC and continue heating. The solution should now be colourless and any remaining sand white. If solution is still coloured, continue heating until this is achieved.
5. Allow contents to cool.
6. Add about 25-ml distilled water and mix well until no more sediment dissolves. Allow to cool and make up to 50 ml with water.
7. Allow to settle so that a clear solution can be taken from the top of the tube for analysis.
8. Determine total N, Ca, Mg, Na, P, Zn, Cu, Fe and Mn in the digests as outlined later in this manual.

Determination of Total Nitrogen

Acid digestion of the plant/soil material is followed by either distillation-titration or by colorimetry. The choice is dependent on local facilities; but colorimetry procedures are more rapid and accurate enough.

Distillation and titration. Free ammonia is liberated from solution by steam distillation in presence of excess alkali (NaOH). The distillate is collected in a receiver (50 ml conical flask) containing excess boric acid with drops of mixed indicator (Figure 9.1).

Figure 9.1. Nitrogen steam distillation apparatus

Reagents

1. Sodium hydroxide, 40% NaOH. Dissolve, carefully, 400 g NaOH in distilled water.
2. N/70 Na_2CO_3: Dissolve 0.1892 g dry Na_2CO_3 in about 250 ml of water make to the mark with distilled water.
3. 100 ppm NH_4-N: Dissolve 0.4714 g of oven dry $(NH_4)_2SO_4$ in distilled water and dilute to 100 ml in a volumetric flask.
4. 1%: Boric acid, H_3BO_3: Dissolve 10-g boric acid in distilled water and dilute to 1000 ml.
5. 0.1N HCl: Dilute 8.1 ml of concentrated HCl (sp gr.1.l) to 1000 ml distilled water.
6. N/70 HCl, exact: Dilute Hydrochloric acid (5) to give N/70 HCl (for plant samples), but N/140 HCl for soil samples.
7. Mixed indicator: Weigh 0.099-g bromocresol green; 0.066 g methyl red and 0.011 g thymol blue, dissolve all with shaking in 100 ml ethanol.

Remarks. Standardise by titrating against 25 ml of sodium bicarbonate (2) N/70 Na_2CO_3 with ml N/70 HCl using methyl orange indicator until colour changes from the pink to magenta end point. The exact normality of the HCl is calculated from the relationship $N_1V_1 = N_2 V_2$. An alternative standardisation of the acid is given in appendix 6 of this manual and subsequent standardisation of sodium hydroxide. 1 ml of acid will be equivalent to 1 mg NH_4-N .(143 ml of 0.1 N HCl diluted to 1litre results in N/70 HCl; 73 ml of 0.1 N HCl diluted to 1 litre results in N/140 HCl). N/70 = 0.014 N HCl. 1-ml of the acid is equivalent to 0.014 meq 1NH_4-N therefore, at equivalent point 0,014* 14mgN= 0.2 mgN/litre (N/140=0.14 N HCl= 0.1mgN/litre). The steam blank should require not more than 0.2-ml acid. A "blank" determination is run by treating the reagents as the sample.

Procedure

1. Set up a steam distillation apparatus (Markham or Hoskyn nitrogen still) and use NH_3-free distilled water wherever possible.
2. Transfer an aliquot (5 ml for plant material, but 10 ml for soils) of sample solution (digest above) to the reaction chamber of the still and add 10 ml of 1% NaOH (Figure 9.2).
3. Steam-distill immediately into 5 ml of 1% boric acid containing 4 drops of the mixed indicator.

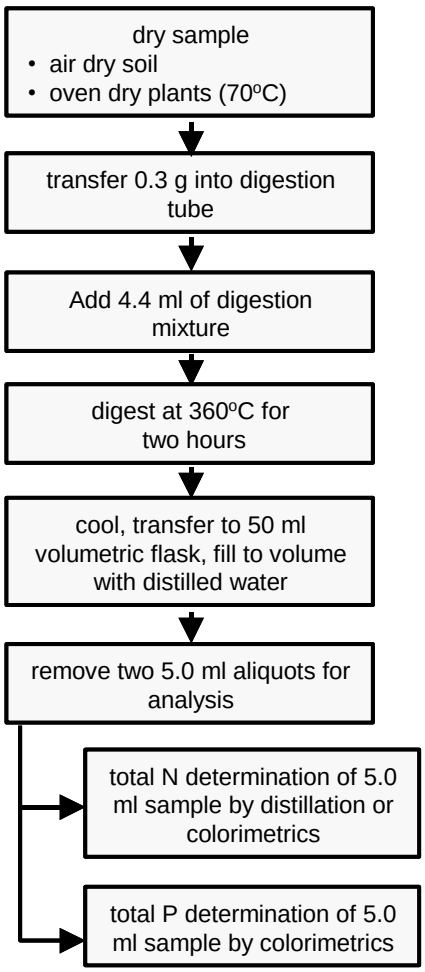

Figure 9.2. A flow diagram of the digestion procedure used to measure total N and P in plant and soil samples.

4. Continue distillation for 2 minutes from the time the indicator turns green.
5. Remove the distillate and titrate with N/70 HCl (for plants), but N/140 HCl (for soils)' the end point being reached when the indicator changes from green through grey to a definite pink. Note ml of the standard HCl required.
6. Pass steam through the apparatus for 30 min. Check the steam blank by collecting 50-ml distillate and titrate with either N/70 or N/140 HCl as given below.
7. Occasionally check that the distillation recovery is satisfactory by taking an aliquot (e.g. 5.0-ml) of the standard ammonium sulphate solution in place of the sample

Calculations

$$\% \text{ N in plant sample} = \frac{(a-b)\ 0.2\times v\ 100}{1000 \times w \times al}$$

$$\% \text{ N in soil sample} = \frac{(a-b) \times 0.1\times v\times 100}{1000 \times w \times al}$$

Where a = volume of the titre HCl for the blank, b = volume of the titre HCl for the sample, v = final volume of the digestion, w = weight of the sample taken and al = aliquot of the solution taken for analysis.

Sample calculation. If the reagent blank titre is 0.05 ml of N/70 in a plant tissue N analysis and weight is 0.3 g, final volume is 50 ml and aliquot is 5 ml. The plant tissue titre is 3.25 ml N/70 HCl, then the corrected ml of N/70 HCl is 3.25 - 0.05 = 3.2 ml and:

$$\% \text{ N in sample} = \frac{(3.20\times 0.2\times 50 \times 100)}{1000\times 0.3\times 5} = \frac{((3.20 \times 0.2)}{0.3} = 2.13\% \text{ N}$$

Colorimetric Determination of Total Nitrogen (in both digest)

Reagents
1. Sodiun citrate
2. Sodiun hydroxide
3. Sodium hypochlorite
4. Sodium nitroprusside
5. Sodium salicylate
6. Sodium tartrate
7. Reagent N1: Dissolve 34 g sodium salicylate, 25 g sodium citrate and 25g sodium tartrate together in about 750-ml water. Add 0.12 g sodium nitroprusside and make up to 1 litre of distilled water. The sample digest solution above is strongly acid. The two colorimetric procedures for P estimates in such solution may be used.
8. Reagent N2: Dissolve 30- g sodium hydroxide in about 750 ml distilled water. Allow to cool. Add 10 ml sodium hypochlorite mix well and make up to 1-litre
9. Stock solution 2500 mgN/litre: Dissolve 11.793 g of ammonium sulphate $(NH4)_2\ SO_4$ in1000ml volumetric flask make up to the mark with distilled water.

Remarks. Reagent N1 and N2 should be made at least 24 hours before use. Store in dark

Standards. In to a clean set of 100-ml volumetric flask containing 200ml water, add 2.5ml digestion mixture. Add 0-1.0-2.0-4.0-5.0-6.0-mls the stock solution (9). The standard series contains 0, 25, 50, 75, 100, 125 and 150 mgN/litre. Dilute the standard series1+9(v/`v) with distilled water, the actual concentration will be 0, 2.5, 5.0, 7.5, 10.0 and 15.0 mgN/litre

Procedure. Dilute all the digest, the blanks to 1+9 (v/v) with distilled water to match the standards. With a micropipette take 0.2-ml sample digest, the blanks in to clearly labelled test tube. Add 5.0 ml of the reagent N1, vortex. Add 5.0 ml reagent N2 and vortex. Allow standing for 2 hour measure the absorbency at 650 nm. The blue is stable for at least 10 hours. Plot a calibration curve and read off the concentration of N in the solution.

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

$$N\ \% = \frac{(a-b) \times v \times 100}{1000 \times w \times al \times 1000}$$

where a = concentration of N in the solution, b = concentration of N in the blank, v = total volume at the end of analysis procedure, w = weight of the dried sample and al = aliquot of the solution taken.

Determination of Phosphorus

Two colorimetric procedures for P measurement may be employed using the digest, the first requires that pH of the digest be adjusted and the second does not.

Total phosphorus procedure with pH adjustment

Reagents

1. Ammonium molybdate/ammonium vanadate mixed reagent: Dissolve 20g ammonium molybdate about in 400 ml of distilled water warmed to about 50°C cool, dissolve 1.0 g ammonium vanadate and in about 300 ml of boiling distilled water, cool, add slowly while stirring, 140 ml of concentrated HNO_3. Mix quantitatively the two solutions and make to1litre mark with distilled.
2. Paranitrophenol, 0.5% w/v - Weigh and dissolve in distilled water 0.5 g p-nitrophenol, make to 100 ml with water.
3. Nitric acid, HNO_3, 1 N: Dilute 63 ml conc. HNO_3 to 1 litre with distilled water.
4. Aqueous ammonia, 6 N NH_3 - Dilute 420 ml of conc. ammonia solution to 1 litre with distilled water.
5. Standard phosphorus stock solution, 1000 ppm P - Weigh 1.0967 g of oven-dry KH_2PO_4; dissolve and make to 250 ml with distilled water (1 ml = 1 mg P).
6. 10 ppm P working solution - Dilute 10 ml of the above stock (1000 ppm P) solution to one litre with distilled water.

Standards. Pipette 0, 5, 10, 15, 20 and 25 ml of the standard 10 ppmP solution into a set of clean 50-ml volumetric flasks, respectively. Treat the standard series through the steps (1-8) below as well as the samples. The vanado-molybdate yellow colour is developed in the standard P solutions contains 0, 1, 2, 3, 4, and 5 ppmP. Note, the standards must be prepared for each batch of samples.

Procedure

1. Pipette 10 ml of the wet-ashed digest solution into a 50- ml volumetric flask.
2. Add 0.2 ml of 0.5% p-nitrophenol indicator solution.
3. Make just alkaline (yellow colour) with 6 N NH_3 solution by drop-wise addition with gentle shaking.
4. Add 1 N HNO_3 drop-wise, with shaking until just colourless.
5. Now add 5-ml ammonium molybdate/ammonium vanadate mixed reagent.

6. Make to 50 ml with distilled water; stopper and mix well.
7. Keep flask for about 30 minutes and measure the absorption of the solution using a suitable colorimeter at 400 nm wavelength setting (yellow colour).
8. Read off the amount of phosphorus present in the solution from a calibration curve.

Total Phosphorus without pH Adjustment Using Ascorbic Acid

Reagents

1. Sulphuric acid, H_2SO_4, 5N: Place one litre clean beaker on asbestos mat (or in cold water in sink). To about 500 ml distilled water, add slowly with stirring, 148 ml conc. H_2SO_4. When cool dilute to 1 litre with water.
2. Ammonium molybdate/antimony potassium tartrate solution - Dissolve 12 g ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$) in 250 ml of warm (50°C) distilled water. Separately dissolve 0.291 g antimony potassium tartrate ($KSb.C_4H_4O_6$) in 100 ml distilled water. Add both solutions to 1000 ml of 5 N H_2SO_4 (above). Mix thoroughly and dilute with distilled water to 2 litres. Transfer to a reagent bottle. Store in a dark, cool place. The mixture keeps for 2 months.
3. Ascorbic acid reducing agent - Dissolve 2.108-g ascorbic acid ($C_6H_8O_6$) in 400 ml of ammonium molybdate/antimony potassium tartrate solution (above) and mix well. This must be prepared as required on the day of analysis (this is adequate for about 30 samples plus the P standards). The solution keeps for about 24 hours. Larger quantities of this reducing agent may be prepared depending on the output of a specific laboratory.
4. Standard phosphorus stock solution, 1000 ppm P: Weigh 1.0967 g of oven-dry KH_2PO_4; dissolve and make to 250 ml mark with distilled water (1 ml = 1 mg P).
5. 10 ppm P working solution: Dilute 10 ml of the standard stock solution above (1000 ppm P) to 1 litre with distilled water.

Procedure. Pipette 5 ml of the supernatant clear wet-ashed digest solution into a 50-ml volumetric flask. Add about 20-ml distilled water to each flask. Add 10 ml of the ascorbic acid reducing agent to each flask, beginning with the standards (see below). Make to 50 ml with water; stopper and shake well. Let stand for 1 hour to permit full colour development. Measure the standards and sample absorbances (blue colour) at 880nm wavelength setting in a suitable colorimeter.

Standards. Pipette 0, 1, 2, 3, 4, 5 and 6 ml of the 10 ppm P working solution (above) into 50 ml volumetric flasks. Add 10 ml of the ascorbic acid reducing solution to each flask. Let stand for 1 hour and read absorbance exactly like the sample solutions above. The standards contain 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm P respectively.

Calculations. Plot a graph of absorbency against standard concentration (above). Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= c in subsequent calculations).

$$\text{P in sample (\%)} = \frac{c \times v \times f}{w}$$

where c = the corrected concentration of P in the sample; v = volume of the digest; f = dilution factor; w = weight of the sample.

With a 10 ml digest aliquot (pH adjustment technique) and a 50 ml final dilution used for colour intensity (absorbency) measurement:

$$\text{P in sample (\%)} = \frac{c \times 0.025}{w}$$

where c = the corrected concentration for sample solution; w = the weight of sample taken (e.g. 0.3 g).

Sample calculation. Prepare a standard graph in which absorbency is plotted on the y-axis and P concentration (ppm) is plotted on the x-axis. Based on the absorbency of a blank sample and a sample, assign a P ppm value to each. For example assuming that the blank = 0.2 ppm and the sample = 4.05 ppm. The corrected P concentration (c) = 4.05 - 0.20 = 3.85 ppm

If a 10 ml digest was used in the colorimetric measurement of P, then:

$$\text{P in sample (\%)} = ((c \times 0.025)/w) = ((3.85 \times 0.025)/0.3) = 0.321\% \text{ P}$$

For a 5 ml aliquot as in the ascorbic acid method without pH adjustment and a 50 ml final dilution used in absorbency measurement:

$$\text{P in sample (\%)} = \frac{c \times 0.05}{w}$$

For a 2 ml aliquot used when the samples contain high levels of P and a 50 ml final dilution was used in absorbency measurement:

$$\text{P in sample (\%)} = \frac{c \times 0.125}{w}$$

Additional Comments on Colorimetric Phosphorus Estimates

- The blue colour is more stable with ascorbic acid reducing agent compared to stannous chloride.
- The *sulphuric/ammonium molybdate/ascorbic acid/antimony/potassium tartrate* single reagent gives a stable blue colour within 10-20 minutes; full development occurs after 1 hour and the colour is stable for 12 hours. This mixed reagent reduces hydrolysation of organic P to inorganic P.
- The antimony catalyses the formation of the phosphomolybdenum-blue complex to its end-point.

General Comments on Sample Wet-Ashing Procedures

- From the standpoint of safety and time, alternative techniques are available (see Digestion Using an Electric Hot Plate and Conical Flasks, below).
- The sample digest may also be brought to 100ml final volume (instead of 50 ml), particularly when large aliquots of the sample digests are needed for multi-nutrient analysis (including micronutrients). Further, near complete quantitative recovery of the digested samples from the tubes/flasks can be achieved through the use of increased quantities of water used to wash containers.
- A precipitate of $CaSO_4$ may be formed on cooling, following the completion of the digestion; it will dissolve within 18-20 hours after the addition of water. Therefore Ca measurement is recommended after this period.
- At the NARC Muguga laboratory, a batch consists of 42 tubes, 36 samples, 2 blanks, 1 plant/soil sample with known low nutrient concentrations (in duplicate) and 1 sample with known high nutrient contents (also in duplicate). These known samples serve as internal quality control (internal standards).

- SiO_2 will dissolve gradually and may then interfere in the determinations. Therefore, the determinations should be done soon after digestion, otherwise the digest has to be filtered. Filtering involves extra cost of filter paper.
- When Zn is to be determined, no rubber stoppers should be used.
- The digestion temperature is set at $150^{O}C$ to avoid frothing for about 1 hour then raised $360^{O}C$ for another 2 hours to complete the digestion.

Digestion Using an Electric Hot Plate and Conical Flasks

Principle. In a laboratory where no block digester is available, one alternative is to carry out wet sample digestion using suitable 'pyrex' glassware on an electric hot plate in a fume hood.

Reagents

1. Sulphuric acid, H_2SO_4, concentrated
2. Hydrogen peroxide, H_2O_2, 30% (or 100 vols).

Procedure

1. Weigh 0.3 g of dry plant or soil sample into a clean dry 125 ml 'pyrex' cornical flask or tall beaker.
2. Add 4 ml concentrated H_2SO_4 and swirl the flask carefully to ensure that all the sample is wetted.
3. Heat, in a fume hood, the flask (and contents) on an electric hot plate set at "medium" heating.
4. Remove flask, cool and add 10 drops of H_2O_2, adding 3-4 drops slowly at a time, to avoid vigorous reaction of the contents.
5. Swirl the flask, keeping contents at the bottom of the flask and reheat, but avoid excessive heating that causes spattering.
6. Cool, add 6 drops of H_2O_2 carefully and reheat.
7. Continue cooling and adding 6 drops of H_2O_2 until there is a change of colour, from black to dark brown.
8. Now turn the heater to 'high' setting on the hot plate and continue cooling, adding 6 drops of H_2O_2 and heating.
9. When solution stays colourless on cooling, add peroxide and leave for the last time on 'high' burner for 10-15 minutes.
10. Cool and transfer contents quantitatively into a 50 ml volumetric flask, using distilled water.
11. Bring contents to the mark with water when cool. Mix contents. This is the solution in which you will determine the N, P, K, Ca and Mg contents, following the procedures given above. Prepare 2 blank digests of the same amounts of reagents (H_2SO_4 and H_2O_2). Correct all data against mean blank value.

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CHAPTER 10

AMMONIUM AND NITRATE-NITROGEN IN SOILS

Principle. Nitrogen is a major element essential for plant growth because it is a constituent of all proteins and nucleic acids. The majority of soil nitrogen resides in organic matter, but this N is continuously being mineralised into NH_4^+ and NO_3^- ions, the forms assimilated by plants. This reflects the need to measure the forms and subsequent movement patterns of these ions in soils during cropping in order to make informed recommendations on the need and rates of N-bearing fertilizers and organic inputs. The method provided below provides measurements of NH_4-N and NO_3-N from a single soil extract. Use of MgO makes the extracts alkaline to enhance NH_4-N determination, while Devarda's alloy reduces NO_3-N to NH_4 that is then readily measured through steam distillation.

NH_4-H and NO_3-N estimates in soil are simultaneously extracted in fresh soils using 0.5 M K_2SO_4 followed by faster accurate colorimetric estimates. The colorimetric method described below provides measurements of NH_4-N and NO_3-N from a single soil extract. Manual procedures are fully described in this chapter. In the colorimetric procedure the nitrites are reduced to nitrates which react with sulphanilamide and α-naphthyl-ethylenediamine dihydrochloride to form a highly colored diazo dye which forms the bases for this analysis.

Apparatus

1. Steam distillation apparatus: 'Pyrex' glass, quick fit, such as the types supplied by Norman Erway Glassbburing, 686, Oak Street, Oregon, W1 53575, USA and by the Cunningham Laboratory, CSIRO, Queensland, Australia
2. Microburette: 5 ml, graduated at 0.01 ml intervals.
3. Shaker: mechanical/electrical.
4. Plastic bottles: 150-200 ml with stoppers.

Reagents

1. Magnesium oxide, MgO. Ignite heavy MgO in an electric muffle furnace for 2 hours at 600-700°C. Cool in a desiccator containing potassium hydroxide pellets and store in a tightly stoppered bottle.
2. Devarda's alloy. Ball-milled (100 mesh) reagent-grade.
3. Potassium chloride, KCl, 2M. Dissolve 1500 g AR grade KCl in 10 litres of distilled water. Store in a plastic container.
4. Sulphuric acid, H_2SO_4, 0.002 N. First prepare 0.1 N H_2SO_4 (2.8 ml concentrated H_2SO_4 in 1 litre of distilled water). Dilute to give 0.002 N H_2SO_4 (20 ml of 0.1 N H_2SO_4 diluted to 1 litre of distilled water provides 0.002 N H_2SO_4).
5. Standardise by preparing 0.002 N sodium carbonate, Na_2CO_3 (0.0265 g dry Na_2CO_3 in 250 ml distilled water). Titrate 25 ml 0.002 N Na_2CO_3 with ml 0.002 N H_2SO_4 using methyl orange indicator until colour changes from the pink to magneta endpoint. The exact normality of the H_2SO_4 is calculated.

Preparing the Indicator Solutions

1. Dissolve 0.30 g bromocresol and 0.165 g methyl red in 500 ml of 95% ethanol.
2. Boric acid, H_3BO_3/indicator solution. Dissolve 20g pure grade H_3BO_3 in about 700 ml of hot distilled water. Cool and transfer to 1 litre volumetric flask. Add 200 ml of 95% ethanol. Add 20 ml of indicator above and shake contents well. Continuously add 0.05 N sodium hydroxide, NaOH (2g NaOH in 1 litre of water) until when 1 ml of the contents treated with 1 ml of distilled water changes from pink to pale green). Dilute the solution to the mark with water.

Nitrogen Standard Solution: Combined Ammonium and Nitrate. Dissolve 0.236 g of oven-dry ammonium sulphate $(NH_4)_2SO_4$ and 0.361 g of potassium nitrate, KNO_3. Dilute to 2 litres with distilled water. The solution contains 25 μg NH_4-N and 25 μg NO_3-N per ml. Store in a refrigerator.

Soil Extraction. Weigh 10.0 g of freshly sampled soil sample (or sample kept in a refrigerator) into a plastic shaking bottle. Add 100 ml of 2 M KCl extracting solution. Stopper and shake contents for 1 hour. Filter through No. 5 or No. 42 Whatman filter paper. If analysis will not be complete in one day, store the filtrate in a refrigerator. Microbial activity, associated with N-mineralization may also be suppressed by storing the extract under refrigerator when the distillation cannot be conducted immediately.

Steam Distillation

1. Set up the steam distillation apparatus above and use NH_3-free distilled water.
2. Pass steam through the apparatus for 30 min. Check the steam blank by collecting 50 ml distillate and titrate with 0.002 N H_2SO_4 as described below. The steam blank should require not more than 0.2 ml acid. Check also the 2 M KCl for possible contamination by steam-distilling 10 ml of this solution.

Measurement of NH_4-N and NO_3-N in soil extracts

1. Add 5 ml of the boric acid indicator solution to a 50 ml conical flask having a calibration of 30 ml.
2. Place the flask under the condenser of the steam distillation so that the end or tip of the condenser is about 40 cm above the surface of the boric acid indicator solution.
3. Pipette an aliquot of 10 ml of the soil extract into the distillation flask and add about 0.2 g (scoop) of ignited (and cool) MgO directly to the bulb of the distillation flask. Attach the flask to the distillation apparatus using the spiral steel springs. Start distillation by closing the stopcock on the steam-by pass tube.
4. When the distillate reaches the 30 ml mark on the receiver conical flask, stop the distillation by opening the stopcock on the steam-bypass tube. Rinse the tip of the condenser with a little distilled water.
5. Determine ammonium-N content in the distillate by titration with 0.002 N H_2SO_4 placed in a micro-burette. The colour change at the end point is from green to a permanent faint pink. At the point of equivalence (end point), 1 ml of 0.002 N H_2SO_4 = 28 μg NH_4-N (using the relationship: Normality x equivalent weight 14 = Number of g/litre).
6. After distilling of the NH_4-N from the sample extract in the above procedure, remove the stopper from the side arm of the distilling flask. Add 0.2 g of Devarda's alloy using a dry powder funnel to reach the bulb of the flask. Replace the stopper immediately into the neck of the side arm. Now distil the NO_3-N (NH_4-N libration) into fresh boric acid contents in another receiving flask. The ammonium is estimated by titration with 0.002 N H_2SO_4 above.

Calculations. The concentration of NH_4-N in the fresh soil sample expressed in μgN/kg is calculated as follows;

$$\mu gN\ kg^{-1}\ \text{in soil} = \frac{(a-b) \times 28 \times v \times MCF \times 1000}{w \times al}$$

where a = titre volume of 0.002 N H_2SO_4 for the sample; b = titre volume for the blank; v = volume of the extracting solution; MCF = moisture correction factor; w = fresh weight of the sample; al = sample aliquot; 28μgN = equivalent to 1 ml 0.002 N H_2SO_4 (Note: 1 ml of 0.002N H_2SO_4 = 28 μg NH_4-N or NO_3-N).

For data correction on soil oven-dry (105°C) basis, the procedure follows.

Moisture determination for data correction

1. Weigh about 1 ± 0.001 g of prepared air-dry material into a dry container of known weight (W1). Record the total weight (W2).
2. Dry at 105°C for 2 hr.

3. Allow to cool in a desiccator and re-weigh (W3).

Calculation

$$\text{dry material (\%)} = \frac{(W3 - W1) \times 100}{(W2 - W1)}$$

Correct all data to a dry weight basis by multiplying by (100 % dry material)

The quantity of NH_4-N or NO_3 in 1 g soil (oven-dry) = ml of 0.002 N H_2SO_4 × 28 × $\frac{W3-W1}{W2-W1}$ µg

Remarks. A reagent blank correction is made by subtracting the ml of 0.002 N H_2SO_4 from soil extract distillate and ml of 0.002 N H_2SO_4 from reagent blank distillate. To monitor accuracy and precision of the method, measurement of the recovery of N from the standard 25 µg (NH_4 and NO_3)-N solution is made through steam followed by titration (0.002 N H_2SO_4) and calculation of concentration of N recovered. This should be done for every batch of soils analysed.

Colorimetric Determination of Ammonium

Reagents

1. Sodiun hydroxide
2. Sodium hypochlorite
3. Sodium nitroprusside
4. Sodium salicylate
5. Determination of Phosphorus
6. Sodium tartrate
7. Reagent N1: Dissolve 34 g sodium salicylate, 25 g sodium citrate and 25 g sodium tartrate together in about 750ml water. Add 0.12g sodium nitroprusside and make up to 1 litre with distilled water. The sample digest solution above is strongly acid. Reagent N2: Dissolve 30g sodium hydroxide in about 750 ml distilled water. Allow to cool. Add 10 ml sodium hypochlorite mix well and make up to 1litre

Remarks. Reagent N1 and N2 should be made at least 24 hours before use. Store in dark
Stock solution 1.000mgN/litre: Dissolve 4.714 g of ammonium sulphate $(NH_4)_2$ SO_4 in1000 ml volumetric flask make up to the mark with distilled water. Standard solution 0.01mg/litre: Dilute 50 g of the above stock in 500 ml with distilled water and make to the mark.

Soil Extraction. Weigh 10.0 g of freshly sampled soil sample (or sample kept in a refrigerator) into a plastic shaking bottle. Add 100 ml of 0.5 M K_2SO_4 extracting solution. Stopper and shake contents for 1 hour. Filter through No. 5 or No. 42 Whatman filter paper. If analysis will not be complete in one day, store the filtrate in a refrigerator. Microbial activity, associated with N-mineralization may also be suppressed by storing the extract under refrigerator when the distillation cannot be conducted immediately. Potassium sulphate 0.5M is used instead of the potassium chloride because of Cl^- ion interfering with the cclorimetric reaction. Freshly sampled soil samples must be used since stored samples may have accumulated nitrate as consequence of continued mineralisation

Standards. Into a clean set of 100-ml volumetric, pipette 0, 5.0, 10.0, 15.0, 20.0 and 5.0 ml the standard solution (100µg NH_4+/ml, above and make up to the mark with 0.5M K_2SO_4. The standard series contains 0, 0.5, 10.0, 15.0, 20.0 and 25.0 µg NH_4-N/ml.

Procedure. With a micro-pipette, take 0.2 ml of the sample extract, the blanks and the standard series into clearly labelled test tubes. Add 5.0ml of the reagent N1and let it stand for at least 15 minutes, vortex. Add 5.0-ml of reagent N2 and vortex. Allow to stand for 1 hour measure the absorbance at 655nm. The blue colour is stable for at least 10 hours. Plot a calibration curve and read off the concentration of NH_4^+-N in the solution. Plot a calibration curve and determine the concentration on the solution.

Calculation. The concentration of ammonium-nitrogen n the oven dry soil expressed in µgNH_4^+-N/kg the is calculated as follows;

$$NH_4\,N\ (\mu g\ kg^{-1}) = \frac{(a\text{-}b) \times v \times MCF \times f \times 1000}{w}$$

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

Colorimetric Determination of Nitrate

Principle. The determination of nitrate follows the extraction of soil in 0.5M K_2SO_4 described earlier in this chapter for ammonium. It is important, however, to note that extraction for the analysis of nitrate should not be done in 2M KCl because of the ionic interference with Cl^-.

Reagents

1. Sodium hydroxide, 4M. Prepare by dissolving160 g sodium hydroxide and dilute to 1 litre with distilled water,
2. Salicylic acid, 5%. Prepare by dissolving 5 g salicylic acid in 95 ml sulphuric acid. Make this reagent at least one day before its use. The reagent remains stable for 7 days if stored in a dark, cool place.
3. Potassium nitrate stock solution, 1000 µg ml NO_3-N. Prepare by placing 7.223 g potassium nitrate that was dried at 105^{o}C and cooled in a desiccator, into a 1000 volumetric flask. Fill to the 1000 ml mark with distilled water.
4. Standard solution, 50 µg ml^{-1} NO_3 N. Prepare by diluting 25.0 ml of the potassium nitrate stock solution in a 500 ml volumetric flask and fill to the 500 ml mark with distilled water.

Standards. Transfer 0, 2.0, 4.0, 6.0, 8.0 and 10.0 ml of the standard solution (50 µg ml^{-1}) into a clean, labelled set of 100 ml volumetric flasks. These are the working standards and contain 0, 2, 4, 6, 8, and 10 µg NO_3-N ml^{-1}. Fill each volumetric flask to the 100 ml mark with 0.5 M potassium sulphate

Procedure. Transfer 0.5 ml of the sample extract, blanks and the standard series K_2SO_4 soil into suitably marked test tube. Add 1.0 ml of salicylic acid to each test tube, mix well and wait for 30 minutes. Add 10 ml 4M sodium hydroxide to each test tube. Mix well and leave for 1hour for a full yellow colour development. The colour is stable for the day. Measure the absorbency at wavelength 419nm. Plot a calibration curve and calculate the absorbency for a particular standard in the series, Read off the value of the samples and the blanks.

Calculation

$$NO_3\,N\ (\mu g\ kg^{-1}) = \frac{(a\text{-}b) \times v \times MCF \times 1000}{w}$$

where a = concentration of NO_3^+-N in the solution, b = concentration of NO_3^+-N the blank, v = volume of the extract; w = weight of the fresh soil; MCF = moisture correction factor. The aliquot taken for both the

standards and the unknown are the same therefore no multiplication factor is required within the calculations.

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CHAPTER 11

EXTRACTABLE SOIL PHOSPHORUS: THE OLSEN METHOD

Principle. The soil is extracted with 0.5 M solution of sodium bicarbonate at pH 8.5. In calcareous, alkaline or neutral soils containing calcium phosphate, this extractant decreases the concentration of Ca in solution by precipitating Ca as $CaCO_3$. The result is an increase of the P concentration in the solution. The Olsen method is suitable for a wide range of soil types and pH values. In acid soils containing Al and Fe phosphate, the P concentration in the solution increases as the pH rises. Precipitation reactions in acid and calcareous soils are reduced to a minimum because the concentrations of Al, Ca and Fe remain at a low level in this extractant. It is to be recognized that many other extraction techniques for plant 'available' phosphorus also exist.

Reagents

1. Sodium bicarbonate, $NaHCO_3$, 0.5 M of pH 8.5. Dissolve 42 g of analytical reagent grade (AR) $NaHCO_3$ in one litre of distilled water. Adjust to pH 8.5 with 1 M sodium hydroxide solution (prepared by dissolving 40 g of AR NaOH in one litre of distilled water). This is the Olsen's extracting solution. Store this solution in a polythene container and check the pH of the solution each month. Five litres of the extracting solution may be prepared by weighing out 210 g of $NaHCO_3$ and buffering to pH of 8.5 with 1 M NaOH.
2. Sulphuric acid, H_2SO_4, 5 N. Place a pyrex beaker on an asbestos mat (or in cold water in a sink). To 500 ml of distilled water contained in this beaker, add slowly with stirring, 148 ml of conc. AR H_2SO_4 (approx. 36 N). When cool dilute to 1 litre with distilled water.
3. Boric acid, H_3BO_3, 0.8 M. Weigh out 49.4 g of AR H_3BO_3 powder and dissolve and dilute to mark (with vigorous shaking) with distilled water.

Ammonium Molybdate/Antimony Potassium Tartrate solution mixed

1. Murphy Riley Solution. Dissolve 12 g ammonium molybdate (AR) in 250 ml of warm (50°C) distilled water. Dissolve separately 0.291 g antimony potassium tartrate (analar) in 100 ml of distilled water. Add both solutions to 1000 ml of 5 N H_2SO_4 (above) which has been transferred to a 2 litre volumetric flask. Mix contents thoroughly and dilute to 2 litres with distilled water. Transfer the contents to a reagent bottle (or Winchester brown bottle). Store in a dark, cool place. The mixture keeps for 2 months.
2. Ascorbic acid reducing agent. Dissolve 1.054 g of ascorbic acid (AR) in 200 ml of the mixed reagent above (ammonium molybdate/antimony potassium tartrate solution) and mix well. This must be prepared as required on the day of the analysis. The solution keeps for about 12 hours only. Increased quantities of this reducing agent may be prepared according to the number of samples to be analyzed.
3. Phosphate standard stock solution, 250 ppm P. Weigh 1.9082 g of oven-dry KH_2PO_4 (AR). Transfer to a clean one litre volumetric flask and dissolve in distilled water to a 1000 ml mark. The concentration of P is 250 mg/litre, 0.250 mg P/ml (i.e. 250 μg P/ml, or 250 ppm P).

Phosphate standard solution. Pipette 0, 1, 2, 5, 10, 15, 20 and 25 ml of the above standard phosphate stock solution (250 ppm P) into clean 500 ml volumetric flasks. Add 100 ml of the Olsen's extracting solution and fill to the 100 ml mark with distilled water. These solutions contain 0, 0.5, 1, 2.5, 5, 7.5, 10 and 12.5 ppm P, respectively.

Procedure

1. Soil extraction (1:20 soil to extractant ratio). Weigh out accurately 2.5 g of air-dry (2 mm) soil into a 150 or 250 ml polythene shaking bottle. Add 50 ml of the Olsen's extracting solution (0.5 M

$NaHCO_3$ pH 8.5) to each bottle. Stopper well and place on a mechanical shaker for 30 minutes. Filter the suspension after shaking through the Whatman No. 42 or 44 paper. Add charcoal if necessary to obtain a clear filtrate. This filtrate is used for the colorimetric P measurements.

2. Colorimetric measurement of phosphorus. Pipette 10 ml of each P standard solution and 10 ml of the sample filtrates and 2 reagent blanks into 50 ml volumetric flasks. Add 5 ml 0.8 M boric acid to each flask. Beginning with the standards and blanks, add 10 ml of the ascorbic acid reagent to each flask. Fill to the 50 ml mark with distilled water. Stopper and shake contents well. After 1 hour, measure the absorbance or transmittance of the solution, at a wavelength setting of 880 nm. Obtain ppm P in solution from the standard P curve. Make corrections for reagent blank P concentration.

Calculations; The concentration of phosphorus in the sample expressed in P mg kg^{-1} is calculated as follows;

$$P\ (mg\ kg^{-1}) = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

where a = the concentration of P in the sample; b = the concentration P in the blank; v = volume of the extracting solution; f = dilution factor; w = weight of the sample.

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CHAPTER 12

SOIL PHOSPHORUS 'AVAILABILITY' INDEX: THE BRAY 2 METHOD

Principle. The combination of HCl and NH_4F is designed to recover easily acid-soluble forms of P, largely the Ca phosphates and a portion of the Al and Fe phosphates. The NH_4F dissolves Al and Fe phosphates by its complex formation with these metal ions in acid solution. In general, the method has been reported widely to be useful on most acid soils. The colorimetric procedure for measuring P proposed here is similar to the one used in Olsen P method.

Reagents

1. Ammonium fluoride, NH_4F, 1 N. Dissolve 37 g of AR NH_4F in distilled water and dilute the solution to one litre. Store the solution in a polythene bottle.
2. Hydrochloric acid, HCl, 0.5 N. Dilute 88.4 ml of AR conc. HCl (11.2 N approx.) to 2000 ml with distilled water.
3. Soil extracting solution, or Bray P2 solution. Add 300 ml of 1 N NH_4F solution and 2000 ml of 0.5 N HCl to 7700 ml of distilled water placed in a 10 or 15 litre container. This gives a solution which is 0.03N NH_4F and 0.1 N HCl. It keeps in glass more than one year.
4. Sulphuric acid, H_2SO_4, 5 N. Place one litre clean beaker on an asbestos mat. To about 500 ml of distilled water, add slowly with stirring, 148 ml of concentrated analytical reagent grade (AR) H_2SO_4 (about 36 N). When cool dilute to 1 litre with distilled water.
5. Boric acid, H_3BO_3, 0.8 M. Weigh out 49.4 g of AR H_3BO_3 powder (AR) into 1 litre volumetric flask. Dissolve and dilute to mark with distilled water.
6. Ammonium molybdate/antimony potassium tartrate solution (mixed reagent). Dissolve 12 g ammonium molybdate (AR) in 250 ml of warm (50°C) distilled water. Dissolve 0.291 g of antimony potassium tartrate in 100 ml of 5 N H_2SO_4. Mix thoroughly and dilute with distilled water to 2 litres. Transfer to a brown reagent bottle. Store in a dark, cool place. The mixture keeps for 2 months.
7. Ascorbic acid reducing agent. Dissolve 1.054 g ascorbic acid in 200 ml of ammonium molybdate/antimony potassium tartrate solution (above) and mix well. This must be prepared as required on the day of analyses. The solution keeps for about 24 hours.
8. 250 ppm P standard stock solution: Dissolve accurately 1.0982 g of oven-dry KH_2PO_4 (AR) into a 1000 ml volumetric flask and fill to the 1000 ml mark with distilled water. The phosphorus content in this solution is 250 mg P per litre or 0.250 mg ml^{-1} (= 250 ppm P)

Standards. Pipette 0, 1, 2, 5, 10, 15 and 20 ml of phosphate standard stock solution (above) into 500 ml volumetric flasks. Add 100 ml of Bray 2 soil extracting solution and fill to the 500 ml mark with distilled water. The standard series solutions contain 0, 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 mg P per litre (ppm P).

Remarks. A one (1) ml of standard stock solution contains 0.250 mg P. In highest standard P solution, for example, 20 ml stock solution diluted in 500 ml distilled water will contain 0.250 × 20 mg P = (5×1000)/500 mg P per litre = 10 ppm P. Some soils that are very high in P may require an additional dilution step before the final analysis is performed**.**

Procedure

1. Weigh 2.50 g of air-dry soil (2 mm) into a 250 ml plastic bottle. Add 50 ml of the Bray 2 extracting solution and shake for 5 minutes. Filter through Whatman No. 5 folded filter (or Nos. 42 and 44 filter). This is the soil extract in which P measurement will be made. If filtrate is not clear, re-filter the solution.

2. Colorimetric measurement of phosphorus. Pipette 10 ml of each P standard series solutions, 10 ml of each soil extract and 10 ml of the blanks into 50 ml volumetric flasks. Add about 20 ml of distilled water and add 5 ml of 0.8 M H_3BO_3. Beginning with the standards, add 10 ml of ascorbic acid reagent to each flask. Fill to the 50 ml mark with distilled water. Stopper and shake contents well. After 1 hour, measure the intensity of the blue colour at 880 nm using a spectrophotometer or colorimeter. Include a reagent blank and a reference sample in your measurements. Boric acid is used to suppress fluoride interference from the extractant.

Calculations. Plot calibration curve of the standard series, concentration against the absorbance of the standard series readings for P in mg P per litre of the solution. If oven dry soil values are required, correct values on basis of a soil moisture determination. The following equation expresses phosphorus as mg P kg^{-1} soil, which is equivilent to parts per million (ppm):

$$\text{P mg kg}^{-1} = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

where a = concentration of P mg l^{-1} in extract solution, b = concentration of P mg l^{-1} in the blank sample, v = extract volume, w = weight of the air dried sample and f = additional dilution factor (optional).

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CHAPTER 13

RESIN-BAG EXTRACTABLE PHOSPHORUS

Principle. Phosphorus recovered within resin-bags is another approach to estimating "available`` P and this chapter provides information on this method. The procedure varies from another resin P extraction method presented in Appendix 2 as these two procedures originate from different sources. The use of anion exchange resins to extract phosphorus from soil was pioneered by Amer *et al.* (1955). They showed that the rate of extraction was dependent on the rate of release of phosphate from the soil into the aqueous medium. In this respect resin bags behave very similarly to roots and many subsequent trials have shown resin-extractable P to correlate better than chemical extractions with plant P uptake. The original method has been simplified and standardized by Sibbensen (1978), whose method is presented here, but with only a few modifications on colorimetric determination of P. The method appears to work on any soil type.

Apparatus. Shaker, wide mouthed 200-250 ml extracting plastic bottles with stoppers, resin-filled bags and glassware

Reagents

1. 0.5 M Sodium bicarbonate. Dissolve 42 g $NaHCO_3$ in 1000 ml of distilled water (deionised water).
2. 0.5 M HCl. Dilute 44.2 ml of concentrated HCl to 1.0 l with distilled water. A ten-litre solution is prepared by diluting 442 ml conc. HCl to 10 litres with water.

Procedure

1. **Resin regeneration.** Place the resin bags in a large clean beaker containing 100 ml of 0.5 M $NaHCO_3$ per bag for 30 minutes, stirring occasionally. Repeat with fresh 0.5 M $NaHCO_3$. Wash the bags twice for 30 minutes with distilled (deionised) water, and store them in the final wash water until use.
2. **Soil sample preparation.** Sieve the soil to pass a 2 mm screen, either dry or fresh soil may be used however better estimates are obtained with fresh soil. If fresh soil is used, then determine the moisture content of a sub-sample so that the results may be expressed on a dry weight basis.
3. **Soil extraction.** Weigh 4.00 g of the sample soil above into an extracting bottle. Add 50 ml of distilled (or deionised) water and a resin bag. Close the bottle and shake for 17 hours on an electric shaker set at a low speed and at room temperature. Discard the soil suspension and rinse the bag in the bottle thoroughly with distilled (deionised) water. Add 50 ml of 0.5 M HCl to elute the P from the bags. When the CO_2 generation has subsided, close the bottle and shake for 30 minutes. Determine the P in the acid elute. No filtration is needed. Repeat step (1) for resin regeneration in preparation for the next extraction (or next set of samples). Include two reagent blanks within each set of soils and at least one standard soil sample.

Colorimetric Estimation of Phosphate: The Murphy-Riley Solution.

Reagents

1. 5 N Sulphuric acid. Add, slowly, 70 ml of analytical reagent grade (AR) concentrated H_2SO_4 to 500 ml of distilled water.
2. Ammonium molybdate. Dissolve 20 g ammonium molybdate in warm (50°C) distilled water and make up to 500 ml. Store the solution in a pyrex glass bottle.
3. Ascorbic acid. Dissolve 1.32 g ascorbic acid in 75 ml distilled (deionised) water. Prepare this reagent **ONLY** when needed.

4. Potassium antimonyl tartrate. Dissolve 0.2743 g potassium antimonyl tartrate in 100 ml distilled (deionised) water.
5. Mixed reagent. Mix 125 ml 5 N H_2SO_4 and 3.75 ml ammonium molybdate. Add 75 ml ascorbic acid solution and 12.5 ml potassium antimonyl tartrate. Prepare **ONLY** when needed. The quantities of these reagents may be doubled according to the size or the number of samples.
6. Phosphate standard stock solution, 250 ppm P. Weigh accurately 1.0982 g of oven - dry analar KH_2PO_4 (potassium dihydrogen orthophosphate). Transfer to a clean one litre volumetric flask and dissolve in distilled water and make to the mark. The P content in this solution is 250 mg/litre or 0.250 mg P/ml (i.e. 250µg P/ml or 250 ppm P).

Phosphate standard solutions. Pipette 0, 1, 2, 5, 10, 15 and 20 ml of the above standard phosphate stock solution (250 ppm P) into clean 500 ml volumetric flasks. Add 40 ml of 0.5 M HCl to each flask and fill up to mark with distilled (deionised) water. This solution now contains 0, 0.5, 1, 2.5, 5, 7.5 and 10 mg P l^{-1} (= ppm), respectively.

Procedure. Measurement of P from soil extracts and P standards. Pipette 10 ml of each P standard solution (above) and 10 ml of the sample elutes and 2 reagent blanks into 50 ml volumetric flasks. Add 5 ml of 0.8 M boric acid to each flask. Beginning with the standards and blanks, add 10 ml of the ascorbic acid reagent to each flask. Make to mark with distilled water. Stopper and shake contents well. After 1 hr, measure the blue colour strengths of the solutions on a spectrophotometer, with a wavelength setting of 880 nm. Read off the ppm P in solution from the standard P curve. Note that when the samples are expected to be high in phosphorous, it important to dilute them before the final analysis commences otherwise you may have to change the factor.

Calculations. The concentration of phosphorus in the sample expressed in P mg kg^{-1} is calculated as follows:

$$\text{P mg kg}^{-1} = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

Where a = concentration of P in elute, b = concentration of P in the blanks, v = volume of the sample, w = weight of the soil sample and f = additional dilution factor.

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CHAPTER 14

PHOSPHORUS SORPTION ISOTHERM

Principle. Phosphorus sorption is the removal of labile P from the soil solution followed by its entry on or into solid phases of the soil. Many tropical soils sorb large amounts of applied phosphates applied as fertilizers that in turn reduce the use efficiency of these materials. Phosphorus sorption is greatest in soils containing oxide and allophanic clay families, followed by kaolinitic and lastly montmorillonitic clays (Sanchez, 1976).

The sorption of P by soils is quantified using sorption isotherms. A sorption isotherm describes the equilibrium relationship between sorbed and dissolved P species at a given temperature. In characterization of soils, the quantity of P required to attain a specific P concentration in an equilibrated soil solution is a useful parameter obtained from the isotherm approach. Phosphorus sorption isotherms are obtained by plotting the values of P sorbed by the soil against the P remaining in the equilibrium supernatant solution (Fox and Kamprath, 1970).

The slope of the curve provides information regarding the buffering capacity of the soil and the intercept at zero P sorption is an estimate of P in soil solution, a value which appears to be related to plant growth. The amount of P required to attain the crop phosphate requirement, 0.2 mg P kg^{-1} soil (ppm P) in the equilibrium solution or any other concentration that has been correlated with optimum crop performance, can be obtained from isotherm of a given soil.

Apparatus

1. Centrifuge, and centrifuge tubes, 50 ml capacity;
2. Reciprocating shaker;
3. UV/Visible spectrophotometer.

Reagents

1. Because of the sensitivity required in this procedure only analytical grade chemicals should be used. These include:
2. Calcium chloride, $CaCl_2$, 0.01 M. Dissolve 1.110 g of $CaCl_2$ in distilled water and fill to 1 litre. But a 10-litre solution is prepared by dissolving 11.10 g $CaCl_2$ with distilled water.
3. Mercuric chloride, $HgCl_2$, 0.1%. Dissolve 0.1 g $HgCl_2$ in 100 ml distilled water. This reagent is used to suppress microbial activity responsible for organic P mineralisation during equilibrium. Caution; $HgCl_2$ is extremely toxic and must be handled and disposed with great care. Less hazardous biostatic reagents that do not contain P may be used at the operators discretion depending upon availability, local regulations and disposal procedures.
4. Stock P solution, 1000 ppm P. Weigh 4.0765 g calcium tetrahydrogen diorthophosphate, $CaH_4(PO_4)_2\ H_2O$. Dissolve and make to 1 litre with 0.01 M Ca Cl_2 solution above.
5. Working equilibrating P solutions. Pipette 10, 20, 30, 40, 50 and 60 ml of the stock (1000 ppm P) solution into 1 litre volumetric flasks. Dilute to marks with 0.01 M $CaCl_2$ solution. Shake well. These working solutions contain 10, 20, 30, 40, 50, and 60 ppm P, respectively.
6. Sulphuric acid, H_2SO_4, 5 N. Place a one litre beaker on an asbestos mat or in a cold water bath. To about 500 ml distilled water, add slowly, with stirring, 148 ml concentrated H_2SO_4. When cool dilute to 1 litre with water.
7. Ammonium molybdate/antimony potassium tartrate solution. Dissolve 12.0 g ammonium molybdate $(NH_4)_6\ Mo_7O_{24}$ in 250 ml warm (50°C) distilled water. Separately dissolve 0.291 g antimony potassium tartrate ($KSbC_4H_4O_6$) in 100 ml distilled water. Add both solutions to 1000

ml of 5 N H_2SO_4 (above). Mix thoroughly and dilute to 2 litres with water. Transfer to a reagent bottle. Store in a dark, cool place. The mixture keeps for 2 months.

8. Ascorbic acid reducing agent. Dissolve 2.108 g ascorbic acid ($C_6H_8O_8$) in 400 ml of ammonium molybdate/antimony potassium tartrate solution (above) and mix well. This must be prepared as required on the day of analysis (this is adequate for about 30 samples plus the standards). The solution keeps for about 24 hours. Larger quantities of this reducing agent may be prepared depending on the output of the laboratory.
9. Phosphate standard stock solution, 250 ppm P. Weigh 1.0982 g of oven-dry potassium dihydrogen orthophosphate, KH_2PO_4, transfer to a one litre volumetric flask and dissolve in distilled water to a 1000 ml mark. The concentration of P is 250 mg/1000 ml, 0.250 mg P ml^{-1} (i.e. 250 μg P ml^{-1}).
10. Phosphate standard solutions. Pipette 0, 1, 2, 5, 10, 15, 20 and 25 ml of the standard phosphate stock solution (250 ppm P) into clean 500 ml volumetric flasks. Fill to mark (500 ml) with 0.01 M $CaCl_2$ solution. Shake well. These solutions contain 0, 0.5, 1, 2.5, 5, 7.5, 10 and 12.5 ppm P, respectively.

Procedure

1. To initiate the soil equilibration with the phosphorus solutions, place 3.0 g air-dried soil that was passed through a 2 mm sieve into a 50 ml centrifuge tube.
2. Add 30 ml of 0.01 M $CaCl_2$ containing known quantities of P from P equilibrating solutions (above) into each tube. Include 2 reagent blanks of 0.01 M $CaCl_2$ containing no P.
3. Add a drop of 0.1% $HgCl_2$ solution to each tube and stopper well and shake on a mechanical/electric shaker for two 30 minute periods daily, for 6 days. Record room temperature during shaking periods daily. Calculate the mean temperature over a 6 day interval to represent the equilibration temperature.
4. On day 6, centrifuge solutions at 7000 rpm until the supernatant liquid is clear. In the absence of a centrifuge the suspension obtained after shaking may be filtered through any of the Whatman No. 542 paper or equivalent. The filtrate is used for the colorimetric P measurements.
5. Pipette 10 ml of the supernatant clear (or filtered) solution obtained after soil P equilibration into a 50 ml volumetric flask. Include also 10 ml of each P standard solution (above) and reagent blank solutions (0.01 M $CaCl_2$). Beginning with the standards and blanks, add 10 ml of the ascorbic acid reagent to each flask and fill with distilled water. Stopper and shake contents well.
6. After 1 hour measure the absorbance or transmittance of the blue coloured solutions on a spectrophotometer, with a wavelength setting on 880 nm. Obtain ppm P in solution from the standard P curve. Make corrections for reagent blank values. The standards now contain 0, 0.1, 0.2, 0.5, 1.0, 1.5, 2 and 2.5 ppm P, respectively.

Table 14.1. Phosphorous additions to soils from the addition of 30 ml equilibrating solutions.

Equilibrating solution (P mg/l)	Quantity of P (mg) added to 3.0 g of soil	Quantity of P added (mg per g^{-1} soil)
0	0	0
10	0.3	0.1
20	0.6	0.2
30	0.9	0.3
40	1.2	0.4
50	1.5	0.5
60	1.8	0.6

Table 14.2. An example P isotherm of a Paleudalf, Muguga, Kenya.[1]

Solution mg P l^{-1}	P added mg g^{-1} soil	P in 5.0 ml mg in 5 ml	P remaining mg P g^{-1} (x/m)	P sorbed mg P g^{-1} soil
0	0	0	0	0
10	0.1	0.01	0.001	0.099
20	0.2	0.02	0.002	0.198
30	0.3	0.04	0.004	0.296
40	0.4	0.08	0.008	0.392
50	0.5	0.14	0.014	0.486
60	0.6	0.26	0.026	0.574

Calculations

Phosphorus sorption parameters are calculated from the P sorption isotherm, using the Langmuir equation:

$$\mathbf{c/(x/m) = 1/[(k \times b) + (c/b)]}$$

where c = final supernatant solution P concentration; x/m = P sorbed per unit soil mass (mgP/g soil); b = absorption maximum (mgP/g soil); k = a constant related to the energy of the soil for phosphorous. A plot of c/(x/m) against c gives a straight line with a slope 1/b and intercept 1/k*b, from which k and b is calculated. The P (mg) that must be added to 3 g soil (see 1 above) is calculated as:

$$\mathbf{(y \times 30)/1000 = 3y/100 \text{ mg P/3g soil}} \text{ and}$$

$$\mathbf{P \text{ (mg) added/1.0 g of soil} = (3y/100)/3 = y/100 \text{ mg P/g soil}}$$

where y = concentration of equilibrating solution in mgP/l or ppm. The external P additions to soil necessary to establish a range of P levels to generate a P isotherm are presented in Table 14.1.

Calculating the supernatant solution P concentration (c). This is obtained in the colorimetric measurement of P in a suitable aliquot (such as 10 ml, above) obtained after 6 day equilibration, followed by centrifugation or filtration (see above). The value is obtained by reading directly from the P standard calibration curve where absorbance or transmittance is plotted against P concentration (mg P l^{-1} = ppm P). For example, to calculate the amount of P in the supernatant solution as mg P g^{-1} soil for a 10 ml aliquot of the supernatant of filtrate used in colorimetric measurement, let the concentration of 10 ml aliquot be c (in P mg/l = ppm). Remember that 30.0 ml equilibrating solution was used for 3.0 g soil. Hence, 10 ml of equilibrating solution was applied to 1.0 g soil. In calorimetric measurement 10 ml (from 1.0 g soil) is diluted to 50 ml, so:

$$\mathbf{P \text{ in supernatant solution} = (c \times 50)/1000 = c/(20 \text{ mg P/g soil})}$$

Following this argument, from a 5.0 ml aliquot:

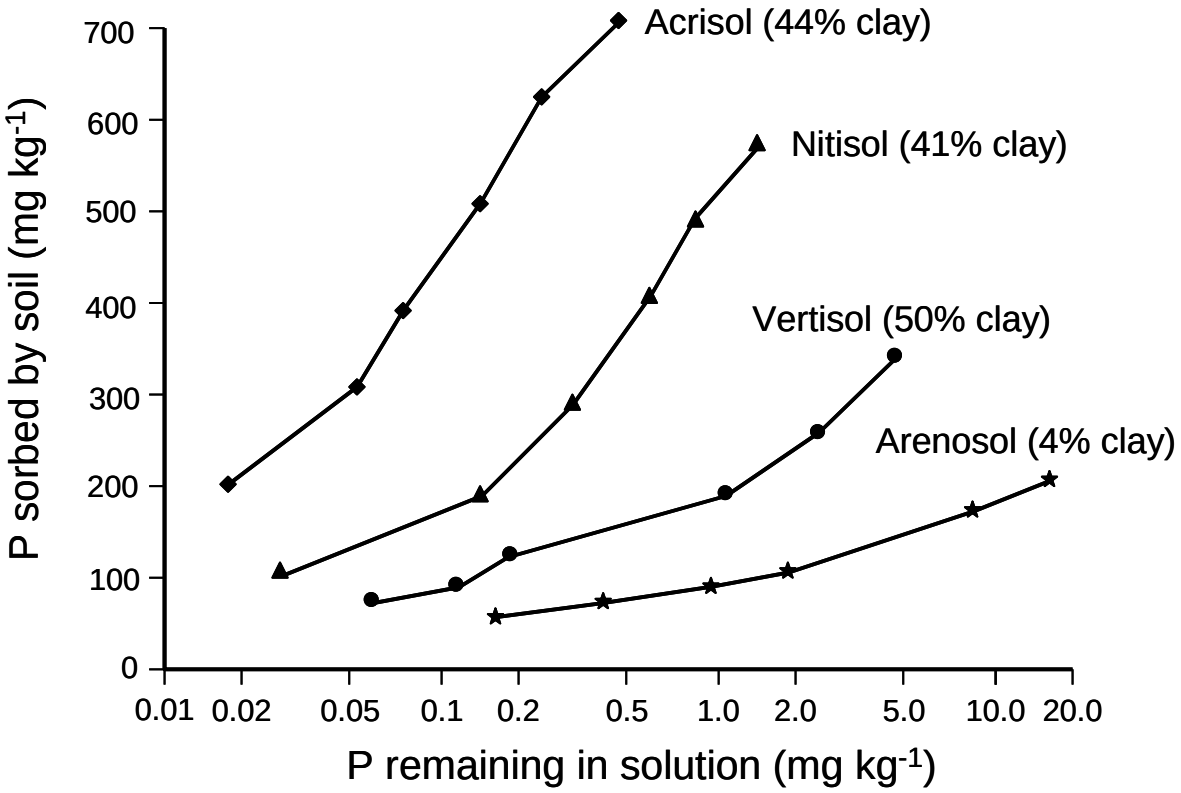

Figure 14.1. Phosphorus sorption isotherms for different Kenyan soils.

P mg/1.0 g soil in supernatant solution = (c × 100)/1000 = c/10

From a 15.0 ml aliquot:

P mg/1.0 g soil in supernatant solution = (c × 33.3)/1000 = c × 0.0333

From a 20.0 ml aliquot:

P mg/1.0 g soil in supernatant solution = (c × 25)/1000 = c/40

Experience at Muguga has shown that light-texture soils, predominantly the luvisols, needed about 5.0 ml aliquot to give a good range of measurements, whereas heavy-textured soils needed larger aliquot of 15 to 20ml. The result of P isotherm procedure are presented for a Kenyan Paleudalf in 14.2.

The P sorption isotherm is plotted with concentration c (mg P l^{-1} = ppm) of P remaining in the supernatant solution on a logarithmic x-axis scale (log_{10}) and P sorbed, x/m, (mg P g^{-1} of soil or ppm) plotted on a y-axis linear scale (Figure 14.1).

Table 14.3. External P levels associated with 95% maximum yield return of selected tropical crops (after Fox *et al.*, 1970).

Crop (*species*)	External P required (ppm)
lettuce (*Lactuca sativa*)	0.40
tomato (*Lycopersicon esculentum*)	0.25
cucumber (*Cucumis sativus*)	0.20
maize (*Zea maize*)	0.60
sorghum (*Sorghum bicolor*)	0.50
cabbage (*Brassica oleracea* var. *capitata*)	0.04

Remarks. Laboratories have different methodology for P sorption measurements, particularly in the ranges of equilibrating solutions and the manner of addition of these solutions, plus shaking time. The standard P concentrations where phosphate requirements are obtained from P sorption curves also vary, e.g. 0, 0.07, 0.2, 0.3 ppm P. Therefore it is suggested that the P requirements should be selected on the basis of crop and information from the literature. Some laboratories have means (e.g. water baths) to maintain the equilibrating temperature constant e.g. at 25°C. However, the important factor here is to record the mean equilibration temperature. The external P levels associated with 95% maximum yield return of selected tropical crops are presented in Table 14.3 (Fox *et al.*, 1970).

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CHAPTER 15

SEQUENTIAL EXTRACTION OF INORGANIC AND ORGANIC PHOSPHORUS POOLS

contributed by Michel A. Beck, North Carolina State University

Principle. Traditional soil P test procedures were developed to improve fertilizer application recommendations. The applicability of these methods to agricultural systems with no or low external P input is limited, particularly in highly weathered soils (Sanchez *et al.*, 1991). The sequential extraction procedure of Hedley *et al.* (1982a) estimates inorganic and organic P fractions. Increasingly harsher treatments extract P pools that are believed to be increasingly less available to plants. Figures 15.1 and 15.2 illustrate represent the modified version of the Hedley procedure presented in this chapter.

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The modifications to filtering, centrifugation speed and temperature improved the method for routine analysis of a large number of samples and had no effect on the distribution of P among the fractions when compared with the original Hedley *et al.* procedure (Beck, 1991). Resin-extractable inorganic P (Pi) has been identified as a pool from which plants readily take up P. $NaHCO_3$ extracts labile inorganic and organic P. NaOH solution extracts moderately labile organic P (Po) and partially dissolves iron and aluminum phosphates and desorbes Pi from sesquioxide surfaces, also termed moderately labile Pi. NaOH-sonicated dissolves moderately labile Pi and Po physically protected by aggregation. HCl dissolves weatherable mineral P and or fertilizer reaction products. Residual P extracted by conc. H_2SO_4 is strongly retained P, unavailable to plants (Hedley *et al.*, 1982b).

Organic P is determined as the difference between total P and inorganic P. Aliquots of the bicarbonate, hydroxide and sonicated hydroxide extracts (containing dissolved organic P) are digested using acidified ammonium persulfate oxidation (EPA, 1971) in the autoclave (103.4 kPa, 121°C for 1.5 hrs). Total P (now all in inorganic form) is then determined on these samples. Inorganic phosphate in the extracts is determined with the molybdate-ascorbic acid procedure of Murphy and Riley (1962).

Reagents

1. Anion exchange resin. Place 0.4 g Dowex 1-X8 >30 mesh, filled into nylon bag (< 30 mesh) measuring approximately 1.5 × 5 cm. Before extraction, the resin is saturated with chloride by soaking the bags for 1 hr in 0.5 M HCl. For use in neutral to calcareous soils, the resin is saturated with HCO_3, using 1 M $NaHCO_3$ instead. Rinse well with distilled water after saturation.
2. Sodium bicarbonate, $NaHCO_3$, 0.5 M, pH 8.5. Dissolve 42 g of analytical reagent grade (AR) $NaHCO_3$ in distilled water and bring to volume of 1000 ml. Adjust pH with 1 M NaOH.
3. Sodium hydroxide, NaOH, 0.1 N. Dissolve 4.00 g of NaOH (AR) in distilled water and make to volume of 1000 ml.
4. Hydrochloric acid, HCl, 1 N. Dilute 83 ml of concentrated HCl (AR) to 1000 ml with distilled water.
5. Sulphuric acid, concentrated.
6. Stock solution for color reagent solution. To 250 ml 5 N H_2SO_4 add 3 g ammonium molybdate and 0.0727 g antimony potassium tartrate. Dissolve and make up to 500 ml. This solution remains stable for weeks.
7. Color reagent solution. Prepare only shortly before use: Take 100 ml of stock solution and dissolve 0.53 g ascorbic acid and stir.

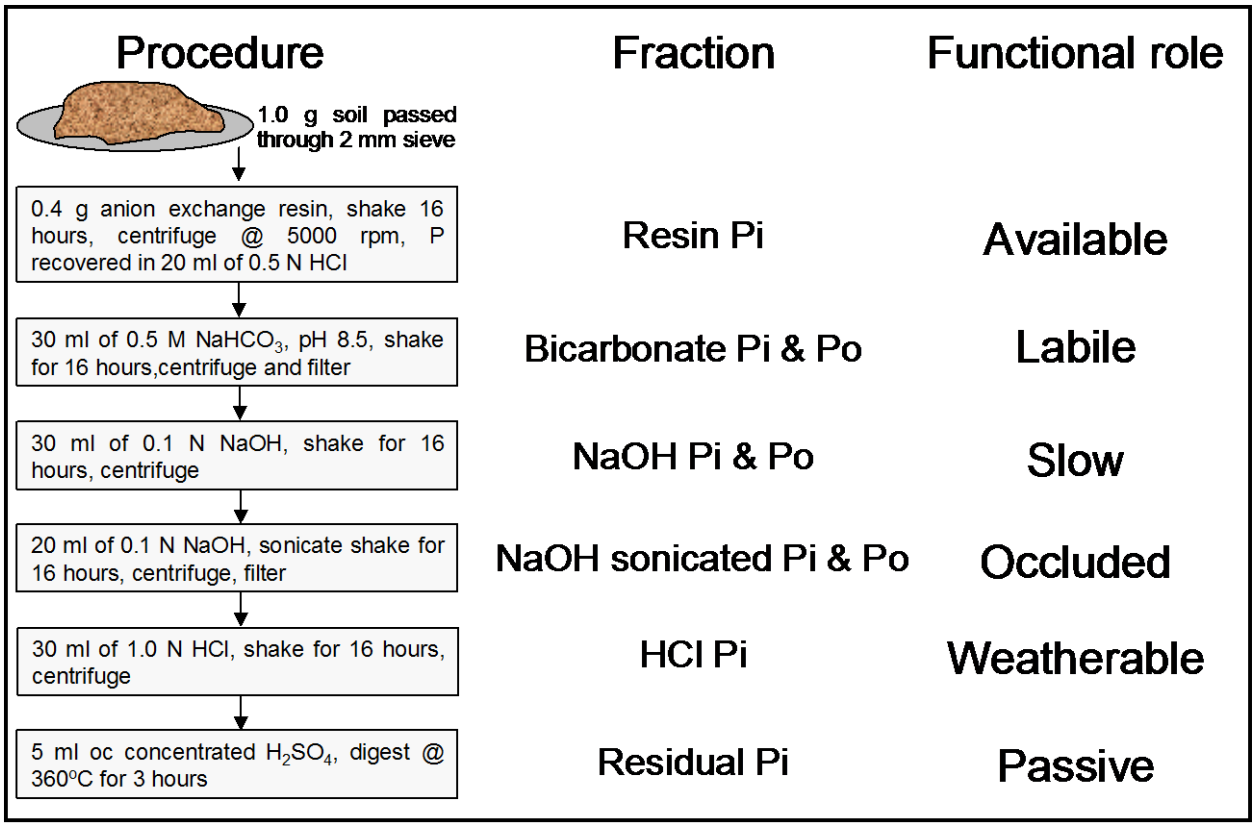

Figure 15.1. A modified Hedley et al. sequential extraction procedure and the functional roles of various P fractions. The extracts are analyzed for total P by acid ammonium persulfate digestion. Within the organic P extraction steps it is assumed that $P_{organic} = P_{total} - P_{inorganic}$.

Procedure

Day 1. Pass soil through 35 mesh (2 mm) sieve and weigh 1.0 g into 50 ml acid washed centrifuge tubes. Rinse resin well with distilled water after acid treatment and add one bag to each tube. Add 30 ml distilled water to each tube, cap and shake overnight for 16 hrs.

Day 2. While removing resin bag with tweezers, wash soil off the bag with distilled water into the tube. Place bags into a matching second set of centrifuge tubes and add 20 ml 0.5 M HCl with a dispenser. Cap and shake for 1 hr. Transfer the bags into distilled water with a few drops of toluene, cover, store in refrigerator until time to regenerate bags for next use. Pipette a 6 ml aliquot into a set of 15 ml vials (calibrated to 9 ml) and determine Resin Pi as follows (Day 2 Procedure):

1. Adjust the pH of the aliquots of the various extracants to near neutral. In general, this is most easily done by calculating how much acid or base is to be neutralized, and counteract that with the respective milliequivalents of base or acid as needed. For example:
2. Resin: 6 ml x 0.5 N HCl = 3 meq H^+; therefore use 0.5 ml of 6 N NaOH to neutralize the acid. This is more convenient than adjusting the pH with an indicator. It is advisable to check the calculation amount of acid or base needed by a simple acid/base titration.
3. Add 1.25 ml of color reagent solution, make to volume (9 ml) with distilled water, and vortex. This will give an acidity of 0.32 - 0.38 meq H^+ for the final solution. This range of acidity is needed for correct color (blue) development.
4. Read on spectrophotometer at 882 nm after 10 mins. Color is stable for at least one hour.

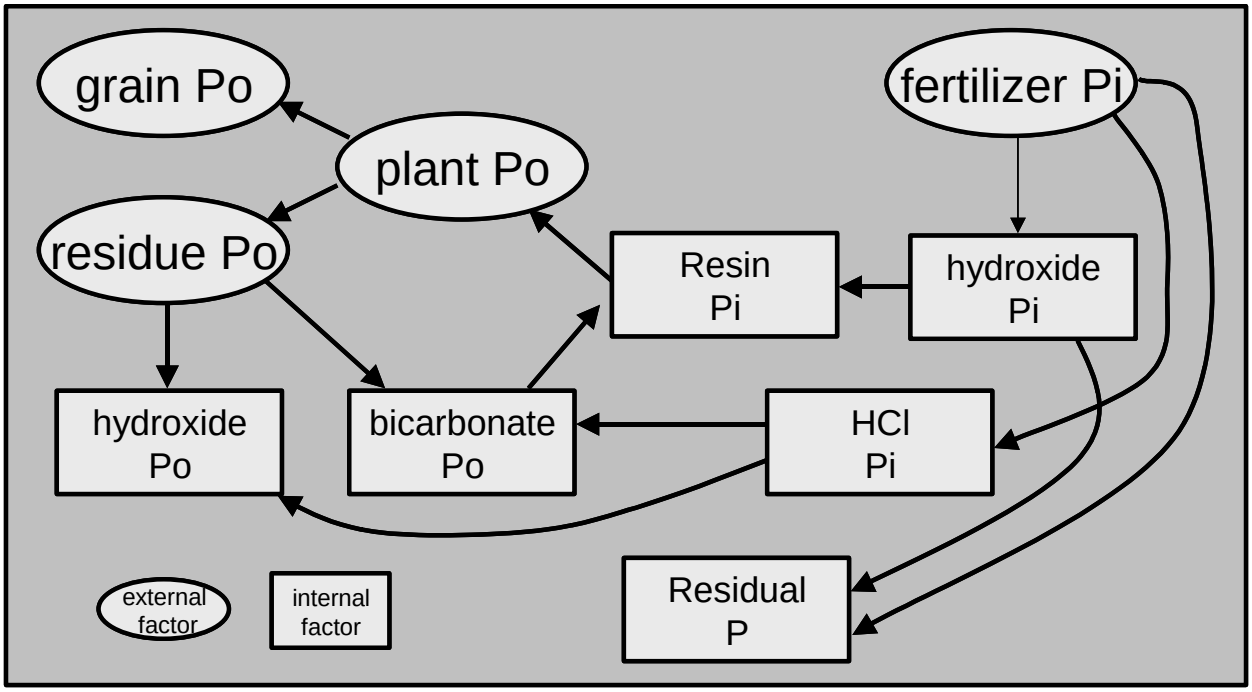

Figure 15.2. Relationships between the labile and stable P pools as fractionated by the Hedley et al. procedure (after Beck and Sanchez, 1994).

Tubes with soil. Centrifuge soil suspension at 2500 to 5000 rpm for 10 min. If solution is not clear, add a small amount of a flocculant like SUPERFLOC and repeat. Decant water carefully as not to lose any soil. Discard water. Dispense 30 ml 0.5 $NaHCO_3$ solution to each tube, cap and shake overnight for 16 hrs.

Day 3. Centrifuge soil suspension as above. Decant $NaHCO_3$ extract into identical set of tubes. Pipette a 5 ml aliquot into glass vials (calibrated to 15 ml). Dispense to each vial 5 ml 2.4 N H_2SO_4 (with 0.5 g $(NH_4)_2S_2O_8$ per 5 ml unit dissolved into the acid just prior to dispensing) and determine $NaHCO_3$ TP as follows (Day 3 procedure).

1. Cover the rack of glass vials with aluminum foil and autoclave for 1 hr @ 15 psi, 120°C.
2. Do not release the pressure, let autoclave depressurize by leaks!!
3. Adjust pH. Example $NaHCO_3$: 5 ml of 0.5 N $NaHCO_3$ = 2.5 meq OH^- (extract aliquot)
   5 ml 2.4 N H_2SO_4 = 12.0 meq H^+ (acid digestion)
   Again, check by titration. The amount of acid produced by organic matter oxidation may vary depending on dissolved organic matter content in extract.
4. Add 2 ml of color reagent solution, make up to volume (15 ml) with distilled water and vortex.
5. Read on spectrophotometer at 882 nm after 30 min.
6. *Pi determination.* If the extract is dark and causing interference in the spectrophotometer reading (e.g. high dissolved organic content) add to the approximately 25 ml left-over extract 3 ml 6 N HCl to acidify the extract and induce precipitation of the dissolved organics. Centrifuge for 1 min at 2000 rpm. Pipette a 6 ml aliquot into 15 ml vials and determine $NaHCO_3$ Pi (see Day 2). If the extract is only lightly coloured, one can proceed with the pipetting of the aliquot without acidifying and precipitating as was done on Day 2.
7. *Tubes with soil.* Dispense 30 ml of 0.1 M NaOH solution to each tube, cap and shake overnight for 16 hrs.

Day 4. Centrifuge soil suspension as above. Decant NaOH extract into identical set of tubes. Pipette a 5 ml aliquot into glass vials. Dispense to each vial 5 ml 2.4 N H_2SO_4 (with 0.5 g $(NH_4)_2S_2O_8$ per 5 ml unit dissolved into the acid just prior to dispensing) and determine NaOH TP (see Day 3 procedure). Add to the approximately 25 ml left-over extract 3 ml 2.4 N H_2SO_4 to acidify the extract. Centrifuge for 1 min at 2000 rpm. Pipette a 6 ml aliquot into 15 ml vials and determine NaOH Pi (Day 2 procedure). *Tubes with soil.* Dispense 20 ml 0.1 M NaOH solution to each tube, sonicate for 15 sec., cap and shake overnight for 16 hrs.

Day 5. Centrifuge soil suspension as above. Decant NaOH extract into identical set of tubes. Pipette a 5 ml aliquot into glass vials. Dispense to each vial 5 ml 2.4 N H_2SO_4 (with 0.5 g $(NH_4)_2S_2O_8$ per 5 ml unit dissolved into the acid just prior to dispensing) and determine NaOH-sonic TP as was done on Day 3. Add to the approximately 15 ml left-over extract 2 ml 2.4 N H_2SO_4 to acidify the extract. Centrifuge for 1 min at 2000 rpm. Pipette a 6 ml aliquot into 15 ml vials and determine NaOH-sonic Pi in the same manner as on Day 2. Tubes with soil: Dispense 30 ml 1 M HCl solution to each tube, cap and shake overnight for 16 hrs.

Day 6. Centrifuge soil suspension as above. Decant HCl extract into identical set of tubes. Pipette a 6 ml aliquot into 15 ml vials and determine HCl Pi. Again, determine P content as was performed on Day 2. To recover soil residue P, transfer soil from centrifuge tubes into 75 ml block digestion tubes, using as little distilled water as possible (this takes practice and will reduce time to evaporate water down to around 5 ml in all tubes). Slowly add 5 ml concentrated H_2SO_4 and swirl so that suspension is well mixed. Digest in digestion block at 360°C for the time it takes the suspension to turn white, plus 1 hr. Let tubes cool down under a hood, then make up to 75 ml volume with distilled water. Cap and shake well. Remove cap and let sit overnight.

Day 7. Pipette 2 ml into 15 ml vials and determine Residual P using the procedure from Day 2.

Standard curves. It is strongly advised to make up the standard curves with the respective extractant solution into a set of centrifuge tubes, and take these tubes through the same pipetting and pH adjustment steps as the samples. This avoids any uncertainties in terms of dilution ratios between the standards and the samples. If standard curves are prepared as given below, no calculations for sample ppm P values are needed other than converting the absorbance values into ppm by use of a regression equation based on the standard.

Resin Pi:	Standard. curve	use x ml of 1 ppm P stock	
	0	0	
	1	1	
	2	2	Bring up with distilled water
	5	5	to volume of extractant used
	8	8	and follow same steps with
	12	12	the samples.
	15	15	

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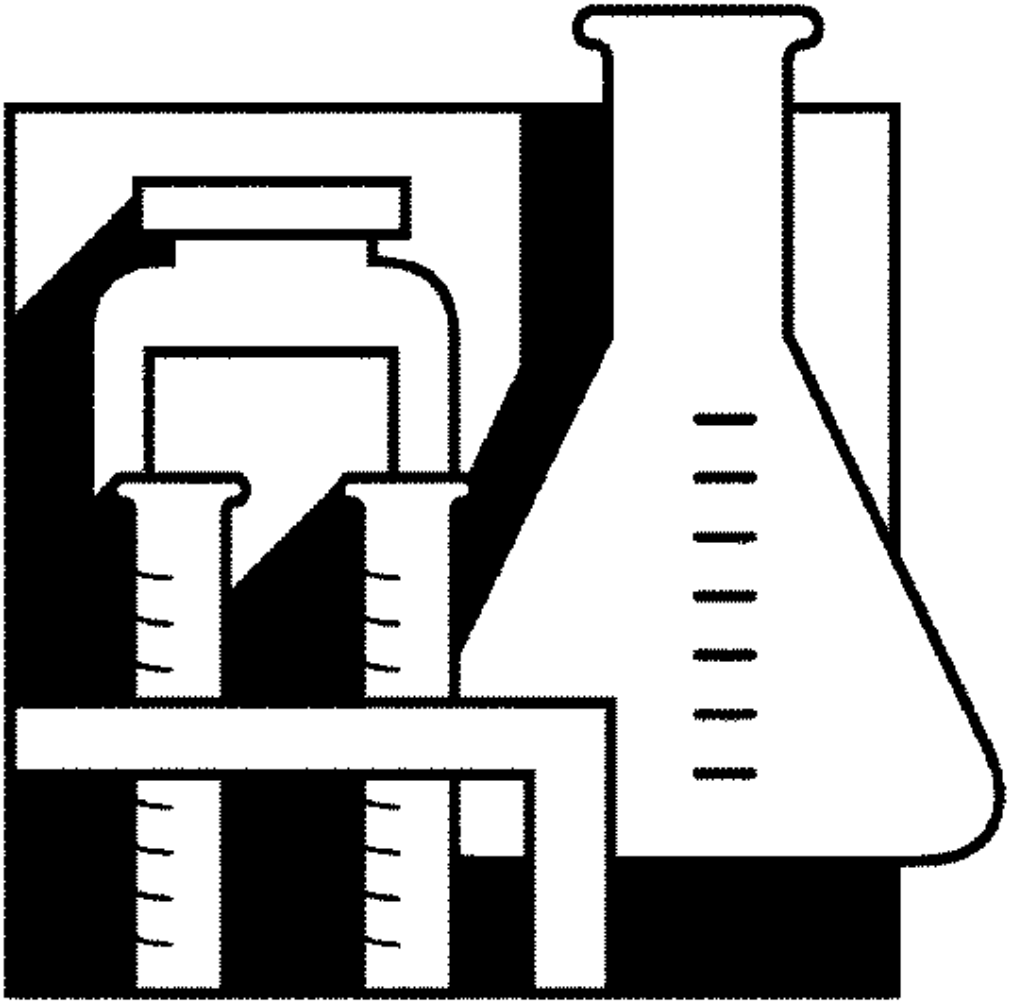

CHAPTER 16

EXCHANGEABLE CATIONS IN SOILS

Principle. A soil sample is extracted with an excess of 1 M NH_4OAc (ammonium acetate) solution such that the maximum exchange occurs between the NH_4 and the cations originally occupying exchange sites on the soil surface. The amounts of exchangeable sodium, potassium, calcium and magnesium in the extract are determined by flame photometry (Na and K) and by atomic absorption spectrophotometry (Ca and Mg). Lanthanum or strontium is added as a releasing agent to prevent formation of refactory compounds, which may interfere with the determinations (e.g. phosphate).

Reagents

1. Ammonium acetate, 1 M NH_4OAc. Dissolve 77.08 g of NH_4OAc in distilled water and make to 1 litre (pH 7) (a larger quantity e.g. 5 litre solution may be prepared by dissolving 385.40 g NH_4OAc to 5 litres). Adjust to pH 7 with acetic acid or aqueous ammonia and mix well. In many cases it may be preferable to prepare this solution from acetic acid and ammonia as these reagents are commercially available in a purer form than NH_4OAc salt which is often contaminated with the elements of interest.
2. 26.8% Lanthanum chloride solution. Dissolve 134 g of lanthanum chloride ($LaCl_3.7H_2O$) in distilled water and make to 500 ml. 1 ml contains 0.1 g La.
3. Potassium standard stock solution, 250 ppm K. Weigh 0.4678 g of KCl dried at $105^{O}C$. Dissolve in distilled water and make to the mark in a 1 litre volumetric flask. This solution contains 250 mg K/1000 ml (250 ppm K).
4. Standard solution, 100ppm K: Dilute 200-ml of the above stock solution (250 ppmK) into a 500-ml volumetric flask with distilled water.
5. Sodium standard stock solution, 250 ppm Na. Weigh 0.6359 g of analytical reagent grade (AR) NaCl dried at 105°C. Dissolve in distilled water and make to the 1 litre mark with distilled water. This solution contains 250 mg Na/1000 ml (250 ppm Na).
6. Standard solution, 100ppm Na: Dilute 200 ml of the above stock solution (250 ppmNa) into a 500ml volumetric flask with distilled water.
7. Calcium standard stock solution, 1000 ppm Ca. Dissolve 2.497 g of dry calcium carbonate (AR), $CaCO_3$, in the minimum quantity of 1:1 hydrochloric acid (AR) and make to 1 litre with distilled water. This solution contains 1000 mg Ca/1000 ml (1000 ppm Ca)
8. Standard solution, 100ppm Ca: Dilute 50-ml of the above stock solution (1000 ppm Ca) into a 500-ml volumetric flask with distilled water.

Standard Solutions

1. Potassium plus Sodium plus Calcium standard solutions. Pipette into clean 100 ml volumetric flasks the following amounts of standard stock solutions:

Addition to flask (ml)	Flask Number #1	#2	#3	#4	#5	#6
Standard K solution	0	1.25	2.5	5.0	7.5	10.0
Standard Na solution	0	1.25	2.5	5.0	7.5	10.0
Standard Ca solution	0	2.5	5.0	10.0	15.0	20.0

2. To each flask add 1 ml of 26.8% lanthanum chloride solution and 10 ml of the 1 M NH_4OAc extraction solution 0, 1.25, 2.5, 5.0, 7.5 and 10 ppm K and Na. The solutions will also contain 0, 2.5, 5.0, 10.0, 15.0 and 20.0 ppm Ca, respectively.
3. Stock solution,100 ppm Mg: Dissolve 1.013-g of magnesium sulphate (AR) $MgSO_4.7H_2O$ and make to the mark with distilled water in a one litre volumetric flask.
4. Strontium chloride solution, $SrCl_2$. Weigh 9.046 g of strontium chloride and dilute to one litre with distilled water. This solution contains 5000 ppm Sr.
5. Magnesium standards. Into a clean set of 100-ml volumetric flasks pipette 0, 1.0, 2.0, 4.0and 5.0 ml of the above magnesium standard stock solution (100 ppm Mg). Add 20-ml of the 5000 strontium chloride $SrCl_2$ (above) and 20 ml of 1 M NH_4OAc extracting solution to each flask. Fill up to the mark with deionised water. These solutions contain 0, 1, 2, 4, and 5 ppm Mg respectively.

Remark: Magnesium sulphate tends to lose crystal water on standing therefore it is to be standardised against EDTA

Procedure

Extraction of Soil. Weigh 5 g of air dry soil (< 2 mm) into a clean plastic bottle with a stopper. Add 100 ml of 1 M (NH_4OAc) ammonium acetate solution (pH 7). Shake contents for 30 minutes and filter through No. 42 whatman paper. This is the soil extract *A* that will be used for Na, K, Ca and Mg determinations (Figure 16.1). Include an internal standard and a repeat sample within each batch of test soils.

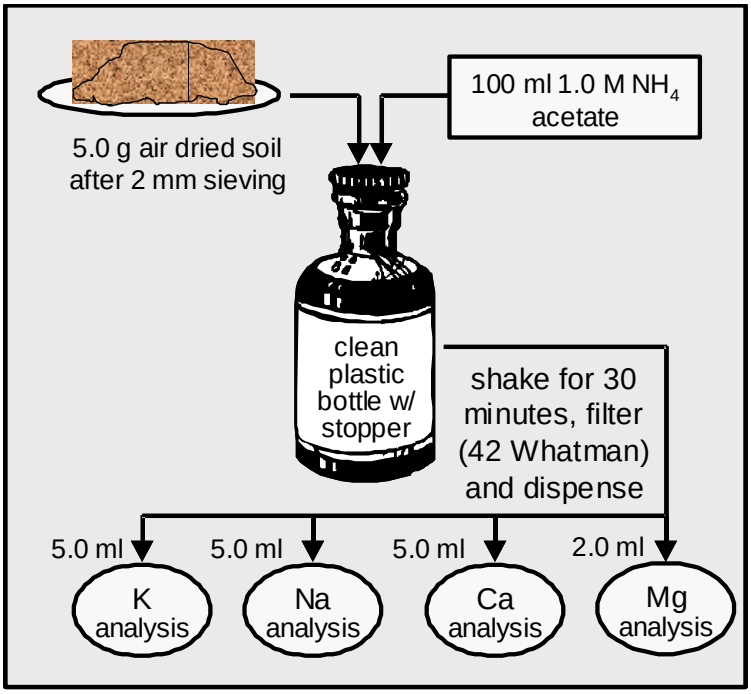

Figure 16.1. Flow diagram of the determination of exchangeable cations in soil.

Determination of Potassium, Sodium and Calcium. To fall within the measurable range of the flame photometer and the atomic absorption spectrophotometer, the soil extract solution A must be diluted ten (10) times for K, Na and Ca determination. Therefore, pipette 5 ml of the soil extract solution A into a 50 ml volumetric flask. Add 1 ml of 26.8% lanthanum chloride solution and dilute the contents to the mark with 1M NH_4OAc extraction solution. Spray this solution into the flame of the flame photometer for the determination of Na and K or into the atomic absorption spectrophotometer flame for Ca measurement. First, the standard working solutions are measured to calibrate the instruments. Note that soil calcium levels are often much higher than the other base cations and the soil extract may require further dilution to place calcium within the measurable range. A similar situation exists for Na in sodic soils.

Determination of Magnesium. The soil extract solution *A* is diluted 25-fold for the determination of magnesium. To make this dilution, pipette 2 ml of the soil extract solution A into a 50 ml volumetric flask. Add 5 ml of 5000 ppm Sr as $SrCl_2$ (above) and fill up to the mark with the 1 M NH_4OAc extracting solution. Spray the solution into the flame of the atomic absorption spectrophotometer. The 25-fold dilution will not always place the Mg content within the appropriate measurable range for all soils and should be altered accordingly.

Calculations. The concentration of K, Na, Ca and Mg in the soil sample expressed in $mgkg^{-1}$ is calculated as follows;

$$mgkg^{-1} \text{ K, Ca, Na and Mg in soil} = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

where a = concentration of K, Na, Ca, and Mg in the sample extract; b = concentration analyte in the blank extract; v = volume of the extract solution; w = weight of the soil sample; f = dilution factor.

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CHAPTER 17

DETERMINATION OF POTASSIUM, CALCIUM AND MAGNESIUM IN PLANT TISSUES

Principle. Total nutrient cation contents of plants and other organic materials may be measured by the complete oxidation of samples using Kjeldahl procedures followed by sprectrometric analysis. Either a flame photometer or atomic absorption spectrophotometer may be used for patassium and magnesium analyses but atomic absorption is necessary for the analysis of magnesium. Standard solutions containing known mixtures of both sodium and the nutrient cations are used because of interference that may occur as a result of mutual excitation between elements.

Plant Sample Digestion

Reagent

Analytical reagent grade ('AR') chemicals are highly recommended

1. Selenium powder, Se
2. Lithium sulphate, $Li_2SO_4.H_2O$
3. Hydrogen peroxide, 30%, H_2O_2 (or 100 volumes)
4. Sulphuric acid H_2SO_4, concentrated
5. Digestion mixture: Add 0.42 g selenium powder and 14 g lithium sulphate to 350 ml 30% hydrogen peroxide and mix well. Slowly add with care 420 ml concentrated H_2SO_4 while cooling in an ice bath. Store at 2°C; the mixture is stable for 4 weeks.

Procedure

1. Weigh 0.3 ± 0.001 g of oven dried (70°C), ground plant tissue into a labelled, dry and clean digestion tube.
2. Add 4.4 ml digestion mixture to each tube and also to 2 reagent blanks for each batch of samples.
3. Digest at 360°C for 2 hours. The solution should now be colourless and any remaining sand white. If solution is still coloured, heat for a further 1 hour. Allow contents to cool.
4. Add about 25 ml distilled water and mix well until no more sediment dissolves. Allow to cool.
5. Make up to 50 ml with water and mix well.
6. Allow to settle so that a clear solution can be taken from the top of the tube for analysis.
7. Determine the K, Ca and Mg in the digests as described below

Determination of Potassium

Reagents

1. Stock Sodium solution, 1000 ppm Na. Weigh 2.541 g of dry (100°C, 2 hr) sodium chloride (NaCl), dissolve in distilled water and make to 1 litre. Store in a reagent bottle.
2. Working Sodium solution, 100 ppm Na. Dilute 20 ml of the above stock solution to 200 ml (this is solution I).
3. Stock Potassium solution, 1000 ppm K. Weigh 1.907 g dry (100°C, 2 hr) potassium chloride. Dissolve and make to 1 litre with distilled water. Store in a reagent bottle.
4. Working Potassium solution, 100 ppm K. Dilute 20 ml of the above stock solution to 200 ml (this is solution II).
5. Sulphuric acid, 1N H_2SO_4.. Carefully dilute 28.0 ml of concentrated H_2SO_4 to 1 litre with distilled water.

Potassium and Sodium Standards. Pipette working Na (I) and K (II) solutions to obtain a seven-step standard series as follows:

1. 5 ml of I plus 10 ml of II into 100 ml flask, giving 5.0 ppm Na and 10 ppm K
2. 4 ml of I plus 8 ml of II into 100 ml flask, giving 4.0 ppm Na and 8 ppm K
3. 3 ml of I plus 6 ml of II into 100 ml flask, giving 3 .0ppm Na and 6 ppm K
4. 2 ml of I plus 4 ml of II into 100 ml flask, giving 2.0 ppm Na and 4 ppm K
5. 1 ml of I plus 2 ml of II into 100 ml flask, giving 1.0 ppm Na and 2 ppm K
6. 0.5 ml of I plus 1 ml of II into 100 ml flask, giving 0.5 ppm Na and 1 ppm K
7. 0 ml of I plus 0 ml of II into 100 ml flask, giving 0.0 ppm Na and 0 ppm K

Before making to 100 ml (with distilled water), add 3 ml of I N H_2SO_4 to each flask. The standard solutions are best stored in reagent bottles.

Procedure

Pipette 2 ml of the wet-digested sample solution (above) into a 50 ml volumetric flask. Make to mark with distilled water and mix well. Spray sample solutions starting with standards, the sample and blank solutions directly into the flame of the flame photometer or atomic absorption spectrophotometer (wavelength at 766.5 nm). Read off the amount of potassium present in the solution (c) from the calibration curve prepared by plotting galvanometer (or transmission) readings against potassium concentrations. Follow the operation instructions given for flame photometer or atomic absorption spectrophotometer.

Calculation. The concentration of potassium in the plant sample expressed in percentage is calculated as follows;

$$\textbf{K (\%) in the sample} = \frac{\textbf{(a-b) × v × f ×100}}{\textbf{1000 × w ×1000}}$$

where a = concentration of potassium in the digest; b = concentration of the blank digest; w = the weight of sample; v = volume if the digest solution; f = dilution factor.

Determination of Calcium

Principle. Standard solutions containing known levels of Na, K and Ca are used to suppress interference from mutual excitation between elements

Reagents

1. Stock calcium solution, 1000 ppm Ca. Dissolve 2.497 g dry (100°C, 2 hr) Calcium carbonate ($CaCO_3$) in the minimum quantity of dilute (1N) HCl and make to 1 litre with distilled water.
2. Working calcium solution, 500 ppm Ca. Dilute 50 ml of the above stock solution to 100 ml with distilled water.
3. Stock potassium solution, 1000 ppm K. Weigh 1.907 g dry (100°C, 2 hr) potassium chloride. Dissolve and make to 1 litre with distilled water. Store in a reagent bottle
4. Working potassium solution, 100 ppm K. Dilute 20 ml of the above stock solution to 200 ml (this is solution II).
5. Stock sodium solution, 1000 ppm Na. Weigh 2.541 g of dry (100°C, 2 hr) sodium chloride (NaCl), dissolve in distilled water and make to 1 litre. Store in a reagent bottle.
6. Working sodium solution, 50 ppm Na. Dilute 10 ml of the above Na stock solution to 200 ml with distilled water.

7. Lanthanum chloride, $LaCl_3 \cdot 7H_2O$, 0.15%. Dissolve 1.5 g lanthanum chloride in distilled water and dilute to 1 litre.
8. Sulphuric acid, 1N H_2SO_4.. Carefully dilute 28.0 ml of concentrated H_2SO_4 to 1 litre with distilled water.

Prepare the Calcium standard

1. Pipette 0, 1.0, 2.0, 3.0, 4.0 and 6.0 ml of the 500 ppm Ca solution into six 100 ml volumetric flasks to give standard solutions containing 0, 5, 10, 15, 20 and 30 ppm Ca, respectively.
2. Add to each flask 5 ml of the 100 ppm K solution
3. Add to each flask 5 ml of the 50 ppm Na solution
4. Add to each flask 40 ml of the 0.15% lanthanum chloride solution
5. Add to each flask about 15 ml of the 1 N H_2SO_4 solution
6. Fill the volumetric flasks to the 100 ml mark with distilled water and mix contents. Store the standard solutions in reagent bottles.

Procedure

Pipette 10 ml of the wet-digested sample solution (above) into a 50 ml volumetric flask. Add 10 ml of 0.15% lanthanum chloride. Make to the mark with distilled water. Shake contents well. Spray the standard, blank and sample solutions into the flame of the flame photometer or atomic absorption spectrophotometer at wavelength 422.7. Construct a calibration curve of the standard series readings and read off the concentration of calcium in the sample and blank solutions

Calculation. The concentration of calcium in the plant sample expressed in percentage is calculated as follows;

$$\textbf{Ca (\%)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{100}}{\textbf{1000} \times \textbf{w} \times \textbf{1000}}$$

where a = concentration of calcium in the digest; b = concentration of the blank digest; w = the weight of sample; v = volume if the digest solution; f = dilution factor

Determination of Magnesium

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

Reagents

1. Stock Magnesium solution, 1000 ppm Mg. Weigh accurately 1.000 g of Spec-pure magnesium rod and dissolve in about 30 ml of 1:1 nitric acid HNO_3. Make to one litre with distilled water.
2. Working magnesium standard solution, 50 ppm Mg. Dilute 10 ml of the above stock solution to 200 ml.

3. Sulphuric acid, 1N H_2SO_4. Carefully dilute 28.0 ml of concentrated H_2SO_4 to 1 litre with distilled water.

Prepare the Magnesium Standard. Into a clean set seven 100 ml volumetric flasks, pipette 0, 0.5, 1.0, 2.0, 4.0, 8.0 and10.0 ml of the Mg standard solution (2, above), add 4.0 ml of 1 N H_2SO_4 to each flask before making up to the mark with distilled water. This provides a series of standards containing 0, 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 mg Mg/litre, respectively. Store these solutions in reagent bottles.

Procedure. Pipette 5 ml of the wet-digested sample solution into a 50 ml volumetric flask. Fill to the 50 ml mark with distilled water and mix contents well. Spray the Mg standard series, the blank and sample solutions into the flame of atomic absorption spectrophotometer. Measure the concentration of the magnesium in the in the standard series and sample and the blank solutions. Construct a calibration curve of the standard series readings and read off the concentration of the sample and blank solution.

Calculation. The concentration of magnesium in the plant sample expressed in percentage is calculated as follows;

$$\mathbf{Mg\ (\%)} = \frac{\mathbf{(a-b) \times v \times f \times 100}}{\mathbf{1000 \times w \times 1000}}$$

where a = concentration of magnesium in the digest; b = concentration of magnesium in the blank digest; w = the weight of sample; v = volume if the digest solution; f = dilution factor

Remarks. The diluted sample digests (above) used for magnesium determination are also used for potassium, thereby providing savings in chemicals and time

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CHAPTER 18

EXCHANGEABLE ACIDITY IN SOIL

Principle. In soils of low pH (<5.5) it is not the hydrogen ions (H^+) that operate as a direct constraint to plant productivity, but rather the abundance of toxic cations, primarily Al^{3+} and to a lesser extent Mn^{2+}, It also causes deficiencies in plant nutrients due to an unfavourable rooting environment (Marschner, 1986; Russell, 1973). In most mineral soils of the tropics, the exchangeable acidity in soils between pH 3 and 5.5 is comprised almost entirely of exchangeable aluminum. Because of the strong correlation of exchangeable aluminum, pH and the inhibition of plant productivity, the measurement of exchangeable acidity and its comparison to the effective cation exchange capacity (Al saturation = exchangeable acidity/ECEC) has become an important soil chemical parameter for highly weathered soils of the tropics. Aluminum ions in soil solution exist in a variety of pH dependant forms. At pH 3, aluminum species are dominated by Al^{3+} and as other forms (particularly $Al(OH)_2^+$) up to pH 5.5. Because of this pH dependency on aluminum activities, extractable acidity is determined using unbuffered, neutral salts, in this case KCl (McLean, 1965).

Exchangeable Acidity by the Titration Method

Reagents

1. 1N KCl, potassium chloride: Weigh 74.56 g of KCl analytical reagent grade (AR), dried (105°C) Dissolve and make to 1 litre with distilled water.
2. 0.05 1N NaOH, sodium hydroxide: Weigh 2.00 gm of NaOH (AR). Dissolve and make to 1 litre with distilled water.
3. 0.05N HCl hydrochloric acid: Dilute 4.4 ml of concentrated HCl (AR, specific gravity 1.19) to 1 litre with distilled water.
4. Sodium fluoride solution, NaF: Dissolve 40 g of NaF (AR) in 1 litre of distilled water.
5. Phenolphthalein indicator: Dissolve 0.1 g of the dry Phenolphthalein powder into 100 ml of 95% ethanol.

Procedure

1. Soil extraction with 1N KCl: Weigh 5 g of air-dry soil (2 mm) into a 45-50 ml centrifuge tube. Add 30 ml of 1N KCl solution. Stopper the centrifuge tube tightly with a rubber bung lined with a clean, dry polythene thin sheet. Shake for 1 hour on a mechanical-electric shaker (reciprocal shaker preferred).
2. Centrifuge the contents at 2000 r.p.m. for 15 minutes. Carefully decant off the clear supernatant liquid into a 100 ml clean volumetric flask (filtration may be necessary to remove floating or organic matter).
3. Add another 30 ml of 1N KCl to the same soil sample and shake for 30 minutes, then repeat step 2 (above) and transfer the clear supernatant into the same volumetric flask.
4. Repeat step 3 (above) for the third time and again combine the clear supernatant into the same volumetric flask. Make up the volume to 100 ml mark with 1N KCl solution.
5. Titration for H and Al. Pipette 25 ml of 1N KCl extract into a 250 ml Erlenmeyer (conical) flask (pipette 50 ml if the soil pH is above 5.0), add approximately 100 ml of distilled water.
6. Add 5 drops of phenolphthalein indicator and titrate the solution with 0.05 N NaOH (from a burette) to a permanent pink end point, with alternate stirring and standing. If necessary, add a few more drops of the indicator to replace that adsorbed by the precipitate of $Al(OH)_3$.
7. The amount of base used is equivalent to the total amount of acidity (H + Al) in the aliquot taken.

8. To the same conical flask, add 1 drop of 0.05 N HCl, to bring the solution back to the colourless state and add 10 ml of NaF solution. While stirring the solution constantly, titrate the solution with 0.05 N HCl (from a separate burette) until the colour of the solution disappears. Add 1 or 2 drops of the indicator. If the colour appears, continue adding 0.05 N HCl until the colour disappears and does not return within 2 minutes. The milliequivalents of acid used are equal to the amount of exchangeable Al.
9. Subtract this value from the milliequivalent of total acidity from the base titration to obtain the milliequivalent of exchangeable H.
10. Express the exchangeable H and Al in m.e. per 100 g of soil (air-dry) after subtraction of values obtained from reagent blanks. Note: Al in the KCl extract may also be determined colorimetrically.

Exchangeable Acidity by a "Shortcut" Method

Principle. A shortcut method is presented in the TSBF Handbook of Methods (Anderson and Ingram, 1993). In this case the same extractant and indicator are used, but the requirement for centrifugation is eliminated.

Reagents

1. Potassium chloride, KCl, 1 M. Dissolve 74.56 g of KCl (AR) and make to 1 litre mark with distilled water. A larger quantity of say 5 litres may be prepared by dissolving 372.8 g KCl and making to 5 litres with distilled water. Store the solution in a plastic or glass container.
2. Sodium hydroxide, NaOH, 0.1 M: Dissolve 4 g of analar NaOH and make to 1 litre with distilled water - larger quantities of solution may be prepared as needed, e.g. 5 litre solutions by dissolving 20 g of NaOH.
3. Phenolphthalein solution. Dissolve 1 g of phenolphthalein indicator in 100 ml of ethanol. Shake contents well.

Procedure. Place 10 g of air-dry (2 mm) soil into a plastic 50 ml container. Add 25 ml of 1 M KCl. Stir contents using a clean glass rod. Allow to stand for 30 minutes. Filter through a Buchner funnel and leach with 5 successive 25 ml aliquots of 1 M KCl. Add 5 drops of phenolphthalein indicator solution and titrate with 0.1 M NaOH to the first permanent pink colour of end point. Correct the titration readings for a blank of titration of 150 ml KCl solution.

Calculation

$$\text{Exchangeable acidity (cmol(+)Kg}^{-1}\text{)} = (\text{ml NaOH sample - ml NaOH blank}) \times 10$$

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CHAPTER 19

SULPHUR IN SOILS AND PLANT TISSUES

Principle. Sulfur is an essential element for plant growth and is found in organic and or inorganic compounds. In well aerated soils the majority (>90%) of the sulfur is in organic form and the small inorganic fraction is predominantly as sulfate anion, the form in which sulphur is taken up by the plant roots. It is therefore, important to evaluate both organically bound sulphur and the free sulfate form by analyzing wet-ashed plant material for total sulphur. The following procedure describes the turbidimetric method for sulfate-S analysis.

Reagents

1. Potassium dihydrogen sulphate extracting solution: Dissolve 0.5491 g of KH_2SO_4 in 1litre distilled water
2. 6 M HCl: Dilute 500ml concentrated HCl to 1 litre, with distilled water
3. Barium chloride
4. Gelatin (difco Bacto Gelatin).
5. Gelatin-$BaCl_2$ reagent: Dissolve 0.6 g of gelatin in 200 ml hot distilled water (60-70^oC) and allow the content to stand in the refrigerator at 4^oC for 16 hours. After 16 hours bring the semi gelatitinous fluid to room temperature. Add 2 g of the reagent grade barium chloride and mix the content until the barium chloride is dissolved. Store the solution in a refrigerator. S/1000: dissolve 2.7175 g KSO_4 in 500 ml volumetric flask. Store in pyrex bottle.(potassium sulfate dried at 105^oC c for 1 hour and stored in desiccator over silica-gel).
6. Working standards: - dilute 0, 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 ml of 1000 mg S/litre to 100 ml with distilled water. The standard series contains 0, 5.0, 10.0, 25.0, 50.0, 75.0 and 100.0 mg S/litre

Note: Allow the reagents to stand for two hours before use.

Digestion Procedure for Plant Tissues

1. Dry the ground plant material for 4 hours (or overnight) at 70^oC
2. Weigh precisely 0.500-1.000 g of the oven dry material into a 25 ml silica evaporating basin.
3. Heat the basin in a muffle furnace at 450^OC for 2 hours allowing a slight access of air to assist combustion.
4. Cool, the basin and add 5 ml 6 N HCl, cover it with clock glass.
5. Transfer the basin to a water bath and with the clock glass still covering the basin, digest the contents for 15 minutes.
6. Rinse the clock glass into the basin, evaporate the contents to dryness and continue heating for further 10 minutes.
7. Repeat the digestion and evaporation (steps 6 and 7) a second time.
8. Add 5 ml of 6 N HCl to the dry residue and heat the basin for 5 minutes on the water bath.
9. Transfer the contents quantitatively to 100 ml volumetric flask, cool and dilute to 100 ml with distilled water.
10. Filter the extract through a watchman filter paper No 40 rejecting the first 5-10 drops of the filtrate.
11. Put a reagent blank through steps 4-10

Extraction Procedure for Soils. Weigh precisely 5 g air dried soil sample in to centrifuge tube. Add 25 ml of the extracting solution. Shake for 30 minutes. Filter the suspension through a

whatman filter paper No 42 and determine the SO_4-S in the solution by turbidity method described bellow.

Turbidity Method

1. Pipette 10 ml of the extract into 50 ml volumetric flasks with about 30 ml distilled water.
2. Add 2 ml of Gelatin-Barium chloride solution (5), mix well.
3. Measure the absorbance at 420 nm on UV/Visible spectrophotometer after 30 minutes.
4. Plot a calibration curve and read off the concentration of sulphur in the sample and blank solutions.

Calculation

The concentration of the sulphur in the dried sample expressed in S (mg kg^{-1}) is calculated as follows

$$\textbf{Sulphur (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f}}{\textbf{W}}$$

where a = concentration of S in the solution; b = concentration of S in the mean values of the blanks; v = final volume of the sample digest; w = weight of the sample taken; f = dilution factor

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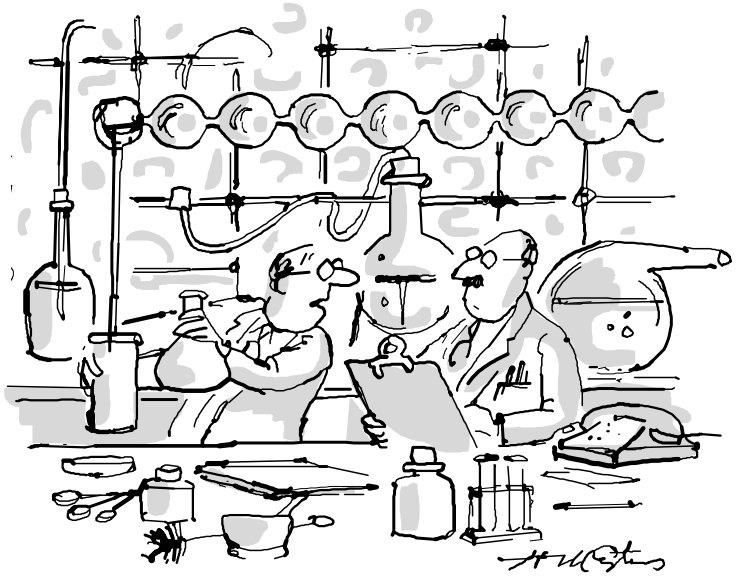

"Quick! Get Alfie on the phone!
I just discovered what it's all about!"

CHAPTER 20

DETERMINATION OF MANGANESE, COPPER, ZINC AND IRON IN PLANT TISSUES

Principle. Micronutrients occupy important metabolic roles in plants, particularly in enzyme systems associated with the synthesis. In some cases the strong ionic charges of these metals serve to bind and configure proteins. Copper, zinc, manganese and iron are four such essential trace elements, with plants occasionally expressing deficiencies in them. In general, these deficiencies are marked by chlorosis or disfiguration of growth tips. To measure these micronutrients, plants and or soil are first digested in a mixture of sulphuric acid, salicylic acid, hydrogen peroxide and selenium powder as outlined in Chapter 9. The digest is then aspirated into a flame from an air-acetylene mixture and detected through a atomic absorption spectrophotometer. See Appendices 8, 9 and 10 for standardization procedures of zinc, copper and iron, respectively.

Sample Preparation and Digestion

Apparatus

1. Aluminium digestion block
2. Atomic absorption spectrophotometer

Reagents

1. Sulphuric acid concentrated (96%)
2. Selenium powder
3. Salicylic acid
4. Hydrogen peroxide 30% (100 volume)
5. Selenium- sulphuric acid mixture: Dissolve 3.5 g selenium powder in 100 ml concentrated sulphuric acid in a beaker. Heat the mixture at 300^{o}C while covering the beaker with a watch glass. The original blackish colour of selenium suspension turns via green/ blue to light yellow.
6. Digestion mixture: Dissolve 7.2 g salicylic acid (3) in 100 ml of the mixture (5). The mixture is stable only 48 hours

Procedure. Weigh 0.3 g of finely ground and dried sample in a dry, clean digestion tube. Take care that all the material settles to the bottom of the tube (does not adhere to the sides of the tube). Add 2.5 ml of the digestion mixture (reagent 6, above) and allow to react at room temperature for at least 2 hours. This analysis is subject to contamination so at least two blanks should be included in each batch of measurements. Heat the tubes in a block digester at 110^{o}C for 1 hour. Remove the tubes from the digester, allow to cool and add three successive portions 1ml of hydrogen peroxide (reagent 4, above), waiting at least 10 seconds between additions. Carefully mix by swirling the tube following each addition. **WARNING: This reaction can be violent, so protective gloves and glasses must be worn.** Return the tube to the block digester and adjust the temperature to 330^{O}C, the colour is brown to yellow. The digestion is complete when the digest becomes colourless or light yellow. Remove the tubes from the block digester and cool to room temperature. Transfer the contents into a 50 ml volumetric flask and make up to the mark deionised water.

Determination of Manganese

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

Reagents

1. 0.8 mol.L^{-1} sulphuric acid: Dilute 45 ml of concentrated sulphuric acid (96%) in a litre of distilled water.
2. Stock solution 500 ppm Mn: Dissolve 1.483 g of potassium permanganate (*Standardise with EDTA, appendix 7*) in about 500 ml of distilled water in a 1000 ml volumetric flask. Reduce the permanganate with a few drops of hydrogen peroxide and make up to the 1000 ml mark with distilled water.
3. Standard solution 50 ppm Mn: Dilute 50 ml of the stock solution (2) in a 500 ml volumetric flask and make the 500 ml mark with 0.8mol litre sulphuric acid

Standards. In a clean set of 100 ml volumetric flasks, pipette 0, 2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 ml of the standard solution (3) and make to the 100 ml mark with 0.8 mol. per litre sulphuric acid (1). This standard series contains 0, 1.0, 2.0, 4.0, 6.0, 8.0 and10.0 Mn mg l^{-1} (ppm), respectively.

Procedure. Prepare the atomic absorption spectrophotometer for use.

1. Switch on the instrument and let it warm for at least 30 minutes.
2. Check to see that acetylene gas tank is not below 75 psi.
3. Adjust the regulators for proper readings according to the equipment specifications AIR and ACETYLENE and proceed.
4. Aspirate the diluted sample, blank digests and the standard series into to the atomic absorption spectrophotometer calibrated for manganese measurement at 248.3 nm and measure the absorbencies. Plot a calibration curve from the absorbencies of the standard series and determine the concentration of the samples

Calculations. The concentration of the manganese in the dried sample expressed in mg $MnKg^{-1}$ is calculated as follows:

$$\textbf{Mn (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Mn in the solution, b = concentration of Mn in the mean values of the blanks, v = volume of the digest; w = weight of the sample taken; f = the dilution factor.

Determination of Copper

Principle. **C**opper is measured by atomic absorption using an element-specific cathode lamp of wavelength of 324.7 nm.

Reagents

1. 0.8 molL^{-1} Sulphuric acid: Dilute 45 ml of concentrated sulphuric acid (96%) in a litre of distilled water.
2. Stock solution of 1000 ppm Cu: Dissolve 3.929 g of copper II sulphate pentahydrate $CuSO_4 \cdot 5(H_2O)$, (*standardised with EDTA, appendix 9*) in 1000 ml volumetric flask. Make up to the 1000 ml mark with distilled water.
3. Standard solution of 50 ppm Cu is prepared by diluting 25.0 ml of the stock solution (2) in a 500 ml volumetric flask. Make up to the 500 ml mark with distilled water.

Standards. In a clean set of 100 ml volumetric flask, pipette 0, 2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 ml of the standard solution (3) and make to the mark with 0.8 mol.L^{-1} of sulphuric acid (1). This standard series contains 0, 1, 2, 4, 6, 8 and 10 mg Cu L^{-1} (ppm)

Procedure. Aspirate suitably diluted sample; blank digests and the standard series into the atomic

absorption spectrophotometer calibrated for copper measurement at wavelength 324.7 nm and measure the absorbencies. Plot a calibration curve from the readings of the standard series and determine the concentration of the unknown

Calculations. The concentration of the copper in the dried sample expressed in Cu $mgKg^{-1}$ is calculated as follows:

$$\textbf{Cu (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Cu in the solution, b = concentration of Cu in the mean values of the blanks, v = final volume of the digestion process, w = weight of the sample taken and f = the dilution factor.

Determination of Iron

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

Reagents

1. 0.8 mol. L^{-1} sulphuric acid: Dilute 45 ml of concentrated sulphuric acid (96%) in a litre of distilled water.
2. Stock solution of 1000 ppm Fe: Dissolve 7.022 g of iron II ferrous ammonium sulphate hexahydrate $Fe(NH_4)_2(SO_4)_2{\cdot}6(H_2O)$ (*standardise with EDTA, appendix 10*) in 1000 ml volumetric flask. Make up to the 1000 ml mark with distilled water.
3. Standard solution of 50 ppm Cu: Dilute 25.0 ml of the stock solution (2) in a 500 ml volumetric flask. Make up to the 500 ml mark with distilled water.

Standards. In a clean set of 100 ml volumetric flask, pipette 0, 2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 ml of the standard solution (3). Make to the 100 ml mark with 0.8 mol l^{-1} sulphuric acid (1). This standard series contains 0, 1, 2, 4, 6, 8 and 10 mg Fe L^{-1} (ppm)

Procedure. Aspirate suitably diluted sample, blank digests and the standard series in to the atomic absorption spectrophotometer calibrated for iron measurement at wavelength 248.3 nm and measure the absorbencies. Plot a calibration curve from the absorbencies of the standard series and determine the concentration of the unknown

Calculations. The concentration of the iron in the dried sample expressed in Fe $mgkg^{-1}$ is calculated as follows;

$$\textbf{Fe (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Fe in the solution; b = concentration of Fe in the mean values of the blanks; v = final volume of the digestion process; w = weight of the sample; f = the dilution factor.

Determination of Zinc

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

Reagents

1. 0.8 mol L^{-1} Sulphuric acid: Dilute 45 ml of concentrated sulphuric acid (96%) in 1000 ml of distilled water.
2. Stock solution of 1000 Zinc: Dissolve 4.398 g of zinc sulphate heptahydrate $ZnSO_4 \cdot 7(H_2O)$ (*standardise with EDTA, appendix 8*) in 1000 ml volumetric flask. Make up to the 500 ml mark with distilled water.
3. Standard solution of 50 ppm Zn: Dilute 25 ml of the stock solution (2) in a 500 ml volumetric flask. Make up to the 500 ml mark with distilled water.

Standards. In a clean set of 100 ml volumetric flask, pipette 0, 1.0, 2.0, 4.0, 8.0 and 10.0 ml of the standard solution (3) and make up to the mark with 0.8 mol.L^{-1} of sulphuric acid (1). This standard series contains 0, 0.5, 1, 2, 4, and 5 mg Zn L^{-1} (ppm)

Procedure. Aspirate suitably diluted sample, blanks digests and the standard series in to the atomic absorption spectrophotometer calibrated for Zinc measurement at wavelength 213.9nm and measure the absorbencies. Plot a calibration curve from the readings of the standard series and determine the concentration of the unknown.

Calculations. The concentration of the Zinc in the dried sample expressed in Zn $mgkg^{-1}$ is calculated as follows:

$$\textbf{Zn (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Zn in the solution; b = concentration of Zn in the mean values of the blanks; v = final volume of the digestion process; w = weight of the sample taken; f = the dilution factor.

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CHAPTER 21

DETERMINATION OF EDTA-SOLUBLE COPPER, IRON, MANGANESE and ZINC IN SOIL

Principle. Copper, zinc, manganese and iron are essential trace elements that plants obtain from soil. While relatively small amounts are required for healthy growth, soil test values serve to predict deficient conditions of soils and to indicate how much is required to ameliorate a given situation (Mergel andKirkby, 1982; Marsch, 1986). Because of the low concentration in soils and plant requirement for micronutrients, it is necessary to accurately determine their levels in soils. Chelating agents such ethylenediaminetetraacetic acid (EDTA) is used in this determination (Vitro, 1955). A suspension of 1% EDTA and soil forms metal-chelate ionic complexes. These complexes, when subjected to an air–acetylene flame in the atomic absorption spectrophotometer are atomised and absorb radiation at element-specific wavelengths. This phenomenon forms the bases for the analysis of these trace elements. See Appendix 6 for standardization procedure of EDTA, and Appendices 8, 9 and 10 for standardization procedures of zinc, copper and iron, respectively.

Sample Preparation and Digestion

Apparatus

1. Reciprocating shaker
2. Atomic absorption spectrophotometer

Reagents. 1% EDTA is prepared by dissolving 10.0 g of the di-sodium salt and diluting to 1000 ml in a volumetric flask with distilled water.

Procedure. Place 5.00 g of air-dried soil (2 mm mesh) in a clean 250 ml plastic bottle fitted with an air-tight screw cap. Add 50 ml of 1% EDTA. Mix the suspension on a reciprocating shaker for 1 hour. Filter the suspension through Whatman filter paper no. 542 or equivalent. The supernatant filtrate constitutes solution A and it is ready for analysis using the atomic absorption spectrophotometer.

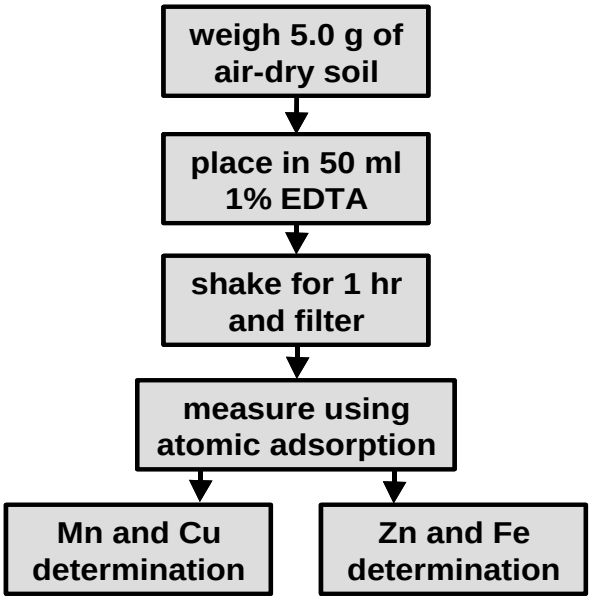

Figure **21.1** Flow diagram for the EDTA extraction procedure and micronutrient analysis of soil.

Determination of Copper

Principle. Air-dried soils are extracted in 1% EDTA, the filtrate is aspirated into an air-acetylene flame of an atomic absorption spectrophotometer, Cu is read at a wavelength of 324.7 nm and the absorbance recorded.

Apparatus. Atomic absorption spectrophotometer.

Reagents

1. Cu stock solution (1000 ppmCu) prepared by dissolving 3.929 g of copper II sulphate pentahydrate ($CuSO_4 \cdot 5(H_20)$, (*standardise with EDTA, appendix 9*), place in a 1 litre volumetric flask and fill to the 1000 ml mark with distilled water.

2. A standard solution 50 ppm Cu is prepared by transferring 25.0 ml of the stock solution and diluting to 500 ml.

Standards. Pipette 0, 2.0, 4.0, 8.0, 16.0 and 20.0 ml of the standard solution into a clean set of 100 ml volumetric flasks. Fill to the 100 ml mark with the 1% EDTA solution. This standard series contains 0, 1, 2, 4, 8, and 10 ppm Cu, respectively.

Procedure. Diluted sample, blank extracts and the standard series is aspirated in the air-acetylene flame of atomic absorption spectrophotometer and the absorbance measured at a wavelength of 324.7 nm. Plot a calibration curve and read the copper concentration in mg Cu litre^{-1} (ppm) of the solution.

Calculation. The content of copper concentration in the air-dried sample expressed in Cu mg kg^{-1} soil is calculated as follows;

$$\textbf{Cu (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Cu in the solution, b= concentration of Cu in the mean values of the blanks, v= final volume of the digestion process and w = weight of the dried sample; f = dilution factor.

Determination of Zinc

Principle. Air-dried soils are extracted in 1% EDTA, the filtrate is aspirated into an air-acetylene flame of an atomic absorption spectrophotometer, Zn is read at a wavelength of 213.9 nm and the absorbance recorded.

Apparatus. Atomic absorption spectrophotometer.

Reagents

1. The stock solution of 1000 ppm Zn is prepared by dissolving 4.398 g of zinc sulphate heptahydrate ($ZnSO_4 \cdot 7(H_2O)$) (*standardise with EDTA*, *appendix 8*). Fill to the 1000 ml mark with distilled water.
2. The standard solution of 50 ppm Zn is prepared by transferring 25.0 ml of the stock solution by pipette into a 500 ml volumetric flask and diluting to 500 ml with distilled water.

Standards. Transfer 0, 1.0, 2.0, 4.0, 6.0 and 10.0 ml of the standard solution by pipette into a clean set of 100 ml volumetric flasks. Fill to the mark with 1% EDTA solution. This series of standards now contain 0, 0.5, 1.0, 2.0, 3.0 and 5.0 ppm Zn, respectively.

Procedure. Diluted sample, blank extracts and the standard series are aspirated in the air-acetylene flame using atomic absorption spectrophotometry and the absorbance measured at wavelength 213.9 mm. Plot a calibration curve and read the zinc concentration of the test solutions in mg Zn per litre (ppm).

Calculation. The content of zinc concentration in the air-dried soil sample, expressed in Zn mgkg^{-1} soil is calculated as follows:

$$\textbf{Zn (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Zn in the solution, b = concentration of Zn in the mean values of the blanks, v = final volume of the digestion process, w = weight of the sample taken and f = the dilution factor.

Determination of Iron

Principle. Air-dried soils are extracted in 1% EDTA, the filtrate is aspirated into an air-acetylene flame of an atomic absorption spectrophotometer, Fe is read at a wavelength of 248.3 nm and the absorbance recorded.

Apparatus. Atomic absorption spectrophotometer.

Reagents

1. Stock solution of 1000 ppm Fe is prepared by dissolving 7.022 g of ferrous ammonium sulphate hexahydrate $Fe(NH_4)_2(SO_4)\cdot 6(H_2O)$, (*standardised with EDTA appendix 10*) into a 1 litre volumetric flask. Fill to the 1000 ml mark with distilled water.
2. Standard solution 50 ppmCu: Pipette 250.0ml of the stock solution (1) and dilute to 500 ml.

Standards. Transfer 0, 2.0, 10.0, 20.0, 40.0 and 60.0 ml of the standard solution into a clean set of 100 ml volumetric flasks by pipette. Fill to the 100 ml mark with the 1% EDTA solution. This series now contains 0, 1, 5, 10, 20 and 30 ppm Fe, respectively.

Procedure. Diluted sample, blank extracts and the standards are aspirated in the air-acetylene flame of the atomic absorption spectrophotometer and the absorbance measured at wavelength 248.3 nm. Plot a calibration curve and read off the iron concentration in mg per litre (ppm) of the solution.

Calculation. The Fe content in the air-dried soil dried sample, expressed in mg Fe per kg soil is calculated as follows:

$$\textbf{Fe (mg kg}^{-1}\textbf{)} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{f} \times \textbf{1000}}{\textbf{1000} \times \textbf{w}}$$

where a = concentration of Fe in the solution, b = concentration of Fe in the mean values of the blanks, v = final volume of the digestion process, w = weight of the sample and f = the dilution factor.

Determination of Manganese

Principle. Air-dried soils are extracted in 1% EDTA, the filtrate is aspirated into an air-acetylene flame of an atomic absorption spectrophotometer, Mn is read at a wavelength of 479.5 nm and the absorbance recorded.

Apparatus. Atomic absorption spectrophotometer.

Reagents

1. Stock solution of 500 ppm Mn is prepared by adding 1.438 g of potassium permanganate $KMnO_4$, (*standardise with EDTA, appendix 7*) to a clean beaker, adding a few drops of hydrogen peroxide to reduce the permanganate, and then transferring the contents to a 1000 ml volumetric flask and filling to the 1000 ml mark with distilled water.

2. Standard solution of 50 ppm Mn is prepared by transferring 50.0 ml of the stock solution into a 500 ml volumetric flask and diluting to 500 ml with distilled water.

Standards. Transfer 0, 2.0, 10.0, 15.0, 20.0 and 30.0 ml of the standard solution into a clean set of 100 ml volumetric flasks by pipette. Fill to the 100 ml mark with 1% EDTA solution. This series of Mn standards contains 0, 1, 5, 7.5, 10 and 15.0 ppm Mn, respectively.

Procedure. **D**iluted soil samples, blank extracts and the series of Mn standards are aspirated into the air-acetylene flame of atomic adsorption spectrophotometer and the absorbance measured at a wavelength of 324.7 nm. Plot a calibration curve and read off the Mn concentration in mg per litre of solution (= ppm).

Calculation. The manganese content of the air-dried soil, expressed in mg Mn per kg of soil is calculated as follows:

$$\mathbf{Mn\ (mg\ kg^{-1}) = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}}$$

where a = concentration of Mn in the solution, b = concentration of Mn in the mean values of the blanks, v = final volume of the digestion process, w = weight of the sample and f = the dilution factor.

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CHAPTER 22

ORGANIC MATTER AND ASH CONTENT OF MANURE AND COMPOST

Principle. The sample is ignited slowly in a muffle furnace to a final temperature of 550°C. The loss in weight represents the moisture and organic matter content of the sample, while the residue represents the ash.

Apparatus. Muffle furnace (550^{o}C capacity)

Procedure

1. Weigh 10 ± 0.1 g of well mixed air dry (< 2 mm) manure or compost sample of a known moisture content in a dry porcelain or nickel crucible.
2. Heat slowly in a furnace (raising the temperature setting in steps (100, 200 and 550°C). The final temperature setting of 550°C should be maintained for 8 hours.
3. Remove the crucible containing a greyish white ash. Cool in a desiccator and weigh.

Calculations. The percentage ash and organic matter are calculated by the differences in weight of the crucibles before and after combustion as follows:

ash (%) = [(W3 - W1) / (W2 - W1)] x 100 and

organic matter (%) = 100 – ash %

where W1 = the weight of the empty, dry crucible; W2 = the weight of the dry crucible containing manure; and W3 = the weight of the dry crucible containing manure following ignition. Note that the weight of the ash = W3 - W1.

Remarks. The percentage of organic matter and ash are usually calculated on oven dry basis of the material. To convert air dried sample ash and organic matter contents from an air dried to a dry weight basis multiply the air dried percentages with:

oven dried sample weight / air dried sample weight

where oven dried sample weight = air dried sample weight x (1 - moisture content).

CHAPTER 23

ORGANIC CARBON CONTENT OF SOILS

Principle. Organic carbon is determined by the sulphuric acid and aqueous potassium dichromate ($K_2Cr_2O_7$) mixture. After complete oxidation from the heat of solution and external heating (Nelson and Sommers, 1975), the unused or residual $K_2Cr_2O_7$ (in oxidation) is titrated against ferrous ammonium sulphate. The used $K_2Cr_2O_7$, the difference between added and residual $K_2Cr_2O_7$, gives a measure of organic C content of soil. The chemical reaction in the method is;

$$\mathbf{2Cr_2O_7^{2-} + 3C + 16\ H^+ \rightarrow 4Cr^{3+}\ 3CO_2 + 8\ H_2O}$$

An additional method is provided where the amount of chromic Cr^{3+}ions formed during the oxidation process is determined colorimetrically to give total amount organic carbon present in soil or manure sample. The method is suitable for soils with higher carbon contents (e.g. >2%).

Reagents

1. 1N Potassium dichromate solution: Dissolve 49.024 g of dry $K_2Cr_2O_7$ in 800 ml of distilled water, and dilute to 1000 ml.
2. Sulphuric acid, concentrated
3. Ferrous ammonium sulphate solution, 0.2 M. Dissolve 78.390 g ferrous ammonium sulphate in 50 ml conc. H_2SO_4, and dilute to 1000 ml with distilled water.
4. Indicator solution: 1,10 Phenanthroline monohydrate - ferrous sulphate (Ferroin). $[C_{12}H_8N_2]_3FeSO_4$. Dissolve 1.485 g of 1,10 ortho-phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$) in 100 ml of 0.025 M ferrous sulphate (0.695 g of ferrous sulphate,
5. $FeSO_4.7H_2O$) in 100 ml of distilled water.

Procedure

1. Weigh out 0.1 to 0.5 g of ground (60 mesh) soil into a block digester tube (sample weight). Add 5 ml potassium dichromate solution and 7.5 ml conc. H_2SO_4.
2. Place the tube in a pre-heated block at 145-155°C for exactly 30 minutes. Remove and allow to cool.
3. Quantitatively transfer the digest to a 100 ml cornical flask, and add 0.3 ml of the indicator solution. Using a magnetic stirrer, to ensure good mixing, titrate the digest with ferrous ammonium sulphate solution; the endpoint is a colour change from greenish to brown.
4. Record the titre and correct for the mean of 2 reagent blanks (T).

Calculation

$$\text{Organic carbon (\%)} = \frac{T \times 0.2 \times 0.3}{\text{sample weight}}$$

***Example Calculation*.** At the titration endpoint (point of equivalence, above):

$$(V_b - V_s)\text{ ml of 0.2 M } Fe^{++}\text{ solution} = (12/4000) \times (0.2 \times (V_b - V_s))\text{ g C}$$

where (Vb - Vs) = T (the titration volume). Hence, the amount of C in a 0.3 g soil sample (w) is:

$$\text{Organic C (\%)} = (0.003 \times 0.2(Vb - Vs) \times 100)/w$$

where V_b = volume in ml of 0.2 M ferrous ammomium sulphate used to titrate reagent blank solution, V_s = volume in ml of 0.2 M ferrous ammomium sulphate used to titrate sample solution and 12/4000 is the millequivelent weight of C in grams. For example, if Vs = 8.75, Vb = 22.75 and w = 0.2 then:

$$C\ (\%) = (0.003 \times (0.2 \times (22.75 - 8.75)) \times 100) / 0.2 = 4.2\%\ C$$

Colorimetric Determination of Organic Carbon

Reagents

1. Barium chloride, 0.4%: dissolve 4 g barium chloride in 1000 ml water.
2. Potassium dichromate, 5%: dissolve 50g in 1000 ml water.
3. Sulphuric acid, concentrated (H_2SO_4, about 36 N)
4. 50 mg/ml C solution: Dissolve 11.886 g dry sucrose in water and make up to 100 ml in a volumetric flask (Dry about 15 g sucrose at 105^OC for 2 hr. Cool in a desiccator)...

Standards. Using a pipette transfer 0, 5, 10, 15, 20 and 25 ml of the 50 mg/ml C stock solution into labelled 100 ml volumetric flask and make up the mark with distilled water, mix well. The standards working series contain 0, 2.5, 5.0, 7.5, 10.0 and 12.5 mg/ml C. Pipette 2 ml of each of working standard into labelled digestion tubes completely dry at 105^oC. The dried content now contains 0, 5, 10, 15, 20, 25, mg C.

Procedure with external heating

1. Weight precisely about 0.30 g ground soil (<0.5mm) into a labeled 100 ml digestion tube. (If the soil is dark, or is suspected to be high in organic matter, use about 0.1 g.) Record the weight of soil, W.
2. Add 2 ml distilled water.
3. Add 10 ml 5% potassium dichromate solution and allow it to completely wet the soil or dissolve the standards.
4. Slowly and carefully add 5 ml H_2SO_4 from a slow burette and gently swirl the mixture to mix.
5. Digest at 150 ^OC for 30 min.
6. Allow to cool, then add 50 ml 0.4% barium chloride, swirl to mix thoroughly, and allow to and bring the volume to 100 ml mark, allow to stand overnight, so as to leave a clear supernatant solution.
7. The standard series now contains 0, 0.05, 0.01, 0.15, 0.20 and 2.5 mg C/ml.
8. Transfer an aliquot of the supernatant solution into a colorimeter cuvette, measure absorbance of the standard, the sample and blank at 600 nm. Record each readings

Calculation

The content of total organic carbon in air dry soil expressed in %C is calculated as follows;
With external heating.

$$\textbf{organic carbon \%} = \frac{\textbf{(a-b) } \times \textbf{0.10)}}{\textbf{w}}$$

Where a = concentration of chromic Cr^{3+} in the sample; b = concentration of chromic Cr^{3+} in the sample; w = weight of soil taken for analysis.

Procedure without external heating

Principle. This procedure is followed when a block digester is not available, rather it relies on the heat generated from the reaction of the soil and the potassium dichromate solution. This procedure tends to strongly underestimate total soil organic C due to incomplete digestion of the soil.

Reagents

1. Redissolve the standard series and proceed as the sample and the blanks
2. Weight about 1 0 g ground soil (<0.5 mm) into a labelled, 100 ml cornical flask. (If the soil is dark, or is suspected to be high in organic matter, use about 0.5 g). Record the weight of soil, (W).
3. Add 10 ml 5% potassium dichromate solution and allow it to completely wet the soil or dissolve the standards.
4. Add 20 ml H_2SO_4 from fast burettes and gently swirl the mixture.
5. Allow to cool, and then add 50 ml 0.4% barium chloride swirl to mix thoroughly bring the volume to 100 ml mark with distilled water and allow to stand overnight, so as to leave a clear supernatant solution.
6. The standard series now contains 0, 0.05, 0.01, 0.15, 0.20 and 2.5 mg C/ml
7. Transfer an aliquot of the supernatant solution into a colorimeter cuvette, and measure and record each standard and sample absorbance at 600 nm.
8. Plot calibration curve of absorbance against standard concentration. Determined solution concentration. Determine solution concentration for each sample and the blanks.

Calculation. The content of total organic carbon in the air dry soil expressed in organic C (%) is calculated as follows.

$$\text{Organic carbon } \mathbf{\%} = \frac{\mathbf{(a\text{-}b) \times 0.1)}}{\mathbf{(w \times 0.74)}}$$

where a = chromic ions (Cr^{3+}) present in the sample; b = chromic ions (Cr^{3+}) present in the blank; 0.74 = correction factor for incomplete oxidation

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CHAPTER 24

FRACTIONATION OF ORGANIC MATTER BY PARTICLE SIZE

Principle. The objective of this procedure is to determine the absolute amounts and relative proportions of particulate organic C and N in soils. Particulate soil organic matter is defined as the fractions with diameters between 50-250 µm and is captured using a wet sieving technique. The assumption is that the particulate fraction is the SOM most readily formed and decomposed in soils and that its levels and dynamics reflect upon the sustainability of various land management options. Furthermore, the particulate fraction is an important substrate for soil mineralisation processes, and a relative decline in the size of this fraction to total SOM is indicative of a loss in inherent soil fertility. The amount of particulate carbon was well correlated with crop yield, soil nutrient content and farmers' perception of soil quality in two studies in Central Kenya (Kapkiyai *et al.*, 1999; Murage *et al*., 2000) and it is likely to prove an efficient index of soil management when employed over a wider range of conditions.

Materials

1. 2 mm, 250 and 50 µm soil sieves
2. Wet sieving apparatus and vibrating sieve shaker
3. 50 g (dw) fresh soil sample
4. Soil dispersal agent (eg 10% Sodium hexametaphosphate (calgon solution), sonication)

Procedure

1. Collect a fresh soil sample and determine the moisture content of a sub-sample
3. Assemble a wet sieving apparatus with mesh sizes 2 mm, 250 and 50 µm.
4. Disperse a 50 g (dw) fresh soil sample with 10% calgon solution. Place the dispersed soil sample on the 2 mm sieve, begin wet sieving.
6.` After 20 minutes, collect the fractions contained on the 2 mm sieve and between the 2 mm -250 µm and 50-250 µm mesh sizes.
7. ***Optional***. Examine selected samples of the 50-250 µm fraction under a microscope at 50 to 100 power to determine the relative proportions of fungal spores and mycelia, plant cellular materials and non-cellular particulate organic matter.
8. Repeat steps 4-7 using 50 g (dw) of a fresh non-dispersed soil sample.
9. Oven-dry the collected samples at 65^{o} C for 24 hours and record the weight of the samples.
11. Determine the C and N contents of the collected materials.
12. Determine the total C and N contents of the soils.

Remarks. Wet sieving of fresh soil is recommended in order to reduce changes in soil structural properties resultant from air or oven drying. The 2 mm, 250 and 50 µm mesh sieves are selected because the particulate organic matter (POM) is defined as having diameters ranging between 50-250 µm (Figure 24.1). The 2 mm mesh collects root litter and coarse to medium sand, the 250 µm mesh collects fine sand and partly degraded plant residues, the 50 um mesh collects silt and POM and passes clay, soil microbes and clay associated organo-mineral complexes (humic substances). This procedure is a rapid approximation of soil organic matter fractions based on particle size only, and does not yield information on microbial biomass. Microscopic examination indicates that both microbial colonies and skins of humic substances adhere to the POM fraction during sieving. Other micro-organisms are fragmented, and very likely washed through to 50 µm sieve.

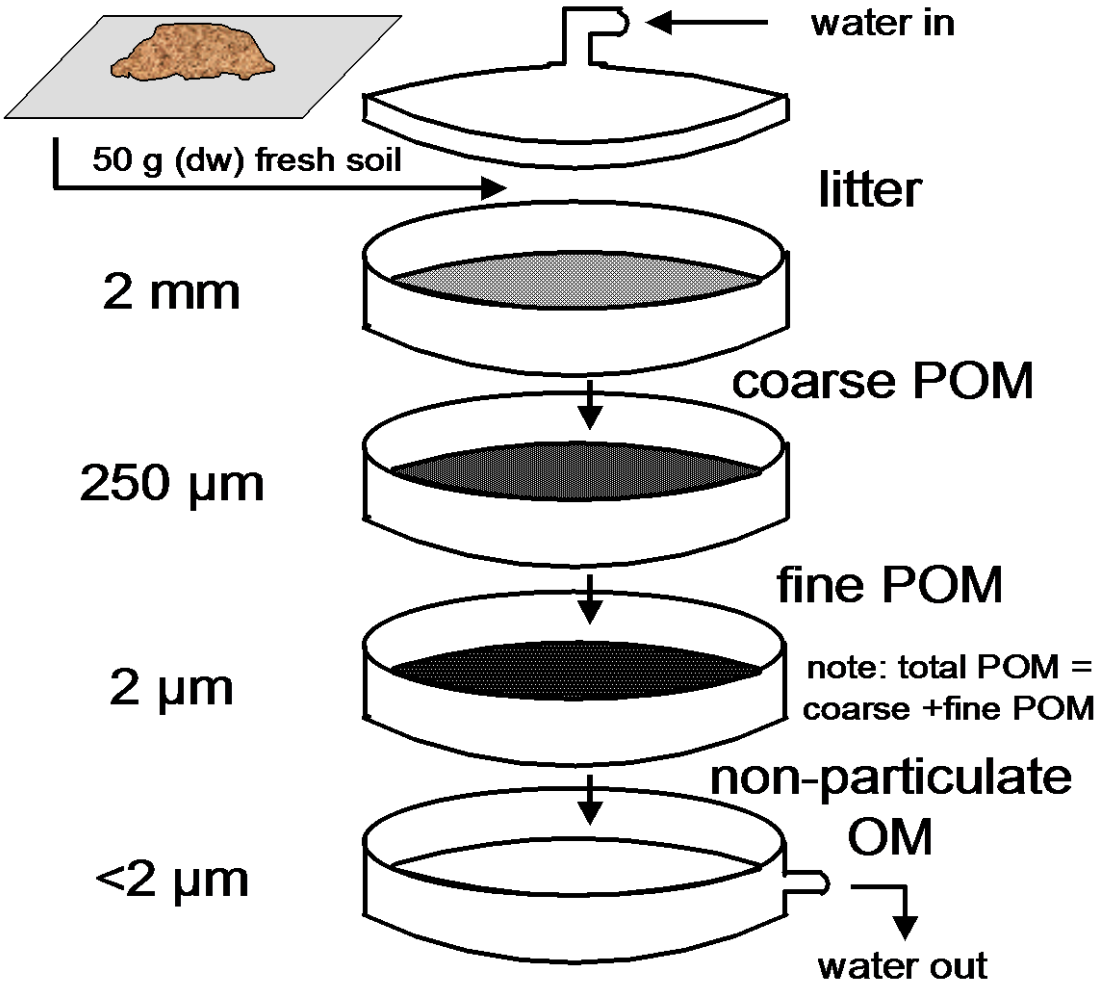

Figure 24.1. Recovery of particulate organic matter and other fractions using the wet sieving technique.

Calculations

1. The total particulate organic carbon of the dispersed soil (POM_T) is:

$$\mathbf{POM_T = (DW_{50\text{-}250}) \times (C\%/100)}$$

2. The unprotected particulate organic carbon of the non-dispersed soil (POM_U) is:

$$\mathbf{POM_U = (DW_{50\text{-}250}) \times (C\%/100)}$$

3. The particulate organic carbon that is physically protected (POM_P) by the soil aggregates is:

$$\mathbf{POM_P = POM_T - POM_U}$$

4. The relative proportion of particulate organic carbon (POM_R) is equal to:

$$\mathbf{POM_R = POM_T / Total\ soil\ C}$$

Note that similar calculations can be performed for the data obtained from the nitrogen analyses. An alternative approach is to compare the N mineralisation of the particulate fraction to that of 50 g (dw) oven dried soil.

Interpretation of Results. The absolute amount of POM is a reflection of the balance between plant residue inputs and the mineralisation of soil organic matter. POM is believed to be a more labile fraction of SOM. Because of this, the relative proportion of POM to SOM (POM_R) is a measure of the mid- to long-term balance of organic matter inputs and losses of a soil system. For example, many forest soils contain greater than 50% POM ($POM_R > 0.5$) and following several years of cultivation and removal of harvest the POM_R drops to < 0.2. The protected particulate organic matter (POM_P) results from the effects of soil aggregation (particularly clays and amorphous minerals) and may represent the longer-term potential of a soil system to provide plant nutrients. In long term plant productivity and soils studies, changes in POM are very likely to be correlated with changes in crop performance over time (Kapkiyai *et al*., 1999) or due to residue management and organic input strategies (Murage *et al*., 2001).

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CHAPTER 25

MICROBIAL BIOMASS CARBON AND NITROGEN

Principle. Microbial biomass as determined by the fumigation-extraction technique subjects a fresh soil to chloroform fumigation that causes cell walls to lyse and denature and the cellular contents to become extractable in 0.5 M K_2SO_4. This is not a measure of soil microbial activity because no differentiation is being made between quiescent and active organisms, or between different classes of microorganisms. Care must be exercised when comparing soils collected from different locations as microbial biomass fluctuates greatly within a single soil in response to litter inputs, moisture availability and temperature.

If different agricultural soils are being compared at a single time, the fresh soils should be at or near moisture holding capacity. If soils from different natural ecosystems are being compared, samples should be collected toward the middle of the wet and dry seasons. The following procedure is based on that of Anderson and Ingram (1993), Brooks et al. (1985) and Vance et al. (1987). Many refinements to measurement of microbial biomass and its components are available, including substrate-induced incubation and the extraction of ATP (see Horwath and Paul, 1994). Powlson (1994) published a concise review of the concept of microbial biomass measurement that contains numerous useful citations.

Reagents

1. Chloroform (alcohol-free). Wash chloroform with 5% concentrated H_2SO_4 in a separation funnel, separate the acid and then rinse repeatedly (8-12 times) in distilled H_2O. Store in a dark bottle.
2. Potassium Sulphate, *0.5 M*. Prepare by dissolving 87.13 g K_2SO_4 in 1000 ml H_2O.

Procedure

1. Place 15 g of fresh soil samples into a 50 ml beaker. Conduct a moisture determination on soil subsamples so that the results can be expressed on a dry weight basis (Figure 25.1).
2. Place the beakers into the two paired desiccators. Into one of the desiccators, place a 100 ml beaker containing 25 ml of chloroform (alcohol-free) into the centre of the desiccator. Adding boiling chips to the chloroform assists in rapid volatilisation of the chloroform. The second desiccator contains the non-fumigated control samples which apart from fumigation/evacuation are to be handled in the same fashion. Close the lids of the desiccators paying particular attention that the sealant is uniformly distributed.
3. Apply a vacuum to the fumigated treatment until the chloroform is rapidly boiling. Close the desiccator and store under darkened conditions for 72 hours at room temperature.
4. Evacuate the fumigated treatments using the vacuum pump repeatedly (8-12 times). Remember that the chloroform is being trapped by the oil in the vacuum pump so the oil must be changed more often than normal. Alternatively, chloroform can be trapped by a cooling finger to prevent contamination of the vacuum oil. It is not necessary to evacuate the control desiccator.
5. Open the dessicators and transfer the soil samples to shaking bottles or flasks (125-250 ml). Add 50 ml of 0.5 M K_2SO_4 and shake on a wrist action shaker for 25 minutes.
6. Either filter the soil suspensions using No 42 filter paper, or centrifuge, in order to obtain a clear extract.
7. Digest the sample as and analyse for total N as described in Chapter 7, and for C as described in Chapter 19.

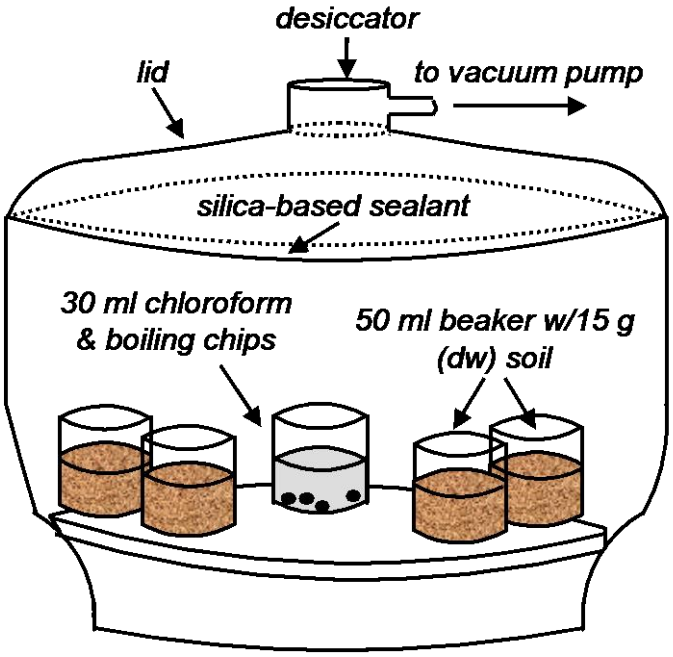

Figure 25.1. Experimental apparatus and sample arrangement in the chloroform fumigation-extraction method for measuring microbial biomass.

Calculations

$$\text{Microbial Biomass C} = (C_{\text{fumigated}} - C_{\text{control}})$$

$$\text{Microbial Biomass N} = (N_{\text{fumigated}} - N_{\text{control}})$$

Remarks. Some authors suggest that empirically derived correction factors should be applied to these results. These factors may be obtained by conducting the fumigation/extraction procedure on inert soils containing a known quantity of microbial biomass (e.g. mushrooms or washed bacterial cells). Vance *et al.* (1987) advocate a factor of 2.64 for microbial biomass C and Brooks *et al.* (1985) recommend a factor of 1.46 for biomass N. If these factors are applied, this should be clearly indicated when reporting the results. Because of the tremendous variation in soil microbial (and microfaunal) populations in soils, we suggest that these factors not be applied.

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CHAPTER 26

PLANT AND LITTER LIGNIN AND CELLULOSE

Principle. The cellulose, lignin and polyphenol contents of organic inputs greatly influence their suitability for use as organic sources of nutrients. Cellulose has an extremely high C to N ratio that may result in short-term immobilisation of soil N following addition of organic materials high in cellulose (Swift *et al*., 1979). Recently developed expert systems (Giller, 2000) and computer simulation models (Parton *et al*., 1994) require information of plant lignin or polyphenolic contents in order to operate. This chapter provides a method, based upon that of Van Soest and Wine (1968) to quantify cellulose, lignin and polyphenolics in acid-detergent fibre. The acid detergent fibre is determined from the plant material by boiling with a sulphuric acid solution of Cetrimethyl Ammonium Bromide (CTAB) under controlled temperature. The CTAB dissolves nearly all the nitrogenous constituents. The acid hydrolyses the starch to leave a residue containing lignin, cellulose and the ash. The lignin is removed by buffered permanganate solution and then cellulose is determined by weight loss upon ashing.

Obtaining the Acid-Detergent Fibre

Reagents

1. Acetone, caution: acetone is highly flammable.
2. Cetyltrimethyl Ammonium Bromide (CTAB)
3. Sulphuric acid (96%)
4. Anti-foam agent
5. 0.5 M sulphuric acid
6. CTAB/ sulphuric solution: Dissolve 1000 g CTAB in 5 litres of 0.5 M Sulphuric acid. The CTAB/sulphuric acid solution may require filtering if cloudy

Reflux Procedure. Weigh accurately 1.0 g (W1) of the plant material into a 250 ml round-bottom flask with a ground glass joint to later fix a reflux condenser. Add 100 ml of the CTAB/sulphuric acid solution and add few drops of anti-foam agent. Connect the condenser and reflux for one (1) hour. Filter through a viteosil crucible (No. 1) of known weight (W2) under gentle suction. Wash the residue with three portions of 50 ml boiling water then wash with acetone to remove all the colour and dry residue fibre. Place the crucible and the content in an oven at 105^{o}C for 2 hours, cool in a desiccator and re-weigh the crucible and the detergent fibre contents (W3)

Determination of Lignin and Cellulose in the Acid-Detergent Fibre

Reagents

1. Acetic acid glacial
2. Ethanol
3. Ferric nitrate
4. Hydrochloric acid
5. Methanol
6. Oxalic acid dihydrate
7. Potassium permanganate
8. Potassium acetate
9. Silver nitrate
10. Silver sulphate
11. Saturated potassium permanganate: Dissolve 50-g potassium permanganate and silver sulphate in water and dilute to 1000-ml with distilled water. Store in dark bottle
12. Lignin buffer: Dissolve 6.0-g ferric nitrate and 0.15 g of silver nitrate. Add 500 ml acetic acid glacial, 5.0-g potassium acetate, 400 ml methanol and dilute to 1000-ml with distilled water. Store in dark bottle.

13. Combined permanganate buffer: Mix reagent (11) and (12) above at the ratio 2:1 by volume (suitable for one week use in dark at 4^0C)
14. Demineralising solution: Dissolve 100 g oxalic acid dihydrate in1400-ml of 95% ethanol. Add 100-ml concentrated hydrochloric acid and dilute to 1litre with distilled water.
15. Ethanol 80%: Dilute 1690-ml of 95% ethanol to 2000 ml with water

Procedure

1. Place the vitrieosil crucible containing the dried acid detergent fibre (ADF) in a shallow enamel pan containing cold water (1 cm depth). Do not wet the fibre at this stage. Add 25 ml of the combined permanganate buffer solution (13, above).
2. Adjust the level of the water in the pan to 2 to 3 cm in order to reduce the flow of the solution from the crucible. Break apart any ADF lumps with a glass rod and spread the contents. Allow to stand for 90 minutes at 20 to 25^{o}C and add more of the combined permanganate buffer solution as necessary to maintain the purple colour.
3. Filter under suction. Place the crucible in clean pan and fill with oxalic acid demineralising solution (14, above). Allow to stand for 15 minutes and the filter under suction. Wash the fibre with demineralising solution until it is white in appearance. Filter and thoroughly wash with 80% ethanol. Filter under suction and repeat twice. Wash similarly with acetone.
4. Dry the crucible for 2 hours at 105^{o}C, cool in a desiccator and weigh (W4). Ash the content at 550^{o}C for 1hour, allow to cool in a desiccator and weigh (W5).

Calculations

$$\textbf{Ash contained in ADF \% = ((W3-W2)/W1) × 100}$$

$$\textbf{Lignin \% = ((W3-W4) / W1) × 100}$$

$$\textbf{Cellulose \% = ((W4-W5) / W1) ×100}$$

Ankom Method: An Alternative Approach For Lignin Analysis

Principle. Lignin is determined via acid detergent fibre. The sample is boiled in sulphuric acid solution of cetrimethyl ammonium bromide (CTAB) under controlled temperature (100^0C). The CTAB dissolves nearly all the nitrogenous constituents and other soluble constituents. The acid hydrolyses the starch to leave a residue containing lignin, cellulose and the ash

Reagents

1. Acetone
2. Cetyltrimethyl ammonium bromide
3. Sulphuric acid (96%)
4. Anti-form agent (Octyl-alcohol).
5. 0.5 M sulphuric acid
6. CTAB/ sulphuric solution: Dissolve 50g CTAB in 5 litres of 0.5 M Sulphuric acid

Procedure by the reflux method

1. Weigh 0.500 to 1.000 g (W1) of the plant material into a 250 ml round-bottom flask with a ground glass joint to fix a reflux condenser.
2. Add 100 ml CTAB/sulphuric acid solution; add few drops of ant-form agent Octyl alcohol.
3. Connect the condenser and reflux for 1hour from the time the boiling starts.
4. Filter through a sintered glass crucible (ASTM 40-60) of known weight (W2) under gentle suction.

5. Wash the residue with at least four portions of 100 ml of boiling water until is acid free (test with blue litmus paper).
6. Wash with an acetone to remove chlorophyll and any other pigment for 1 hour.
7. Air-dry residue fibre by applying suction.
8. Place the crucible and the content in an oven at 105^{o}C for 2 hours, cool to slightly above room temperature and re-weigh the crucible and the content (W3) (Ankom method offers a alternative and quick approach for ADF digestion as described here below. Place the glass crucible containing the dried acid detergent fibre over small beaker.
9. Add concentrated sulphuric acid enough (about 20 ml) to cover the fiber and with stirring glass rod, stir intermittently for every 30 minutes repeat the procedure three times within 2 hours.
10. Wash the fibre with hot water several times, filter under suction.
11. Wash similarly with acetone
12. Dry the crucible and the content for 2 hours at 105^{o}C, cool in a desiccator and weigh (W4).
13. Ash the content at 500-550^{o}C for 1hour, allow to cooling in a desiccator and weigh.(W5).

Calculations

$$\text{Ash-containing ADF (\%)} = \frac{(W3-W2)}{W1} \times 100$$

$$\text{Lignin (\%)} = \frac{(W3-W4)}{W1} \times 100$$

$$\text{Cellulose (\%)} = \frac{(W4-W5)}{W1} \times 100$$

ADF procedure by Ankom Fiber Analyser

Procedure. Ankom filter bags (*ANKOM corp-#F57*) contains negligible moisture however, after standing the bags may require to be redried. To prepare the bags:

1. Weigh filter bag and record the weight (W1) (*QNKOM Corp-# F 57*), zero balance
2. Weight 0.5 gm ± 0.01 gm of sample directly into filter bag. (W2)
3. Seal the bag within 1cm from the open edge using a heat sealer. Spread sample uniformly inside the filter bag. This is done by shaking and light flicking the bag to eliminate clumping.
4. Place 24 bags in the bag suspender. The bag Suspender is composed of nine individual baskets, one center post and a weight. Place three bags per basket; 24 bags total. Stack basket on center post with each basket rotated 120 degrees. The 9th basket remains empty and acts as a top for the 8th basket. The weight is placed on top of the 9th basket to keep the bag Suspender submerged.
5. Add 2000 ml of ambient temperature extracting solution into digestion vessel **NOTE:** When solution level is reduced to 1600ml, ADF reflux time may need to be reduced by approximately 5 minutes to adjust for the faster heat up circle.
6. Place bag suspender with samples into the solution in vessel. Adjust timer for 60 minutes and push START. Turn Agitation and Heat ON, Confirm agitation and then screw down vessel top.
7. When indicated by timer, turn heat and agitation OFF, open the valve and exhaust hot solution before opening lid. **WARNING:** *The solution in the digestion vessel is under pressure. Therefore the valve needs to be opened first in order to release the pressure before the vessel lid can be opened. Ensure that the effluent hose is properly positioned and secured for safe disposal*.

8. After the solution has been exhausted close valve and open the lid. Add approximately 2 litre of hot rinse water and turn Agitator ON and leave the heat OFF. Close the lid but do not tighten. Agitate the bags in rinse water for 3-5 minutes. Repeat hot water rinse four more times.
9. Remove filter bags from bag suspender and gently press out excess water.
10. Place bags in a 250 ml beaker and add 200 ml of acetone to cover bags. Allow bags to soak for 2 or 3 hours, remove and lightly press out excess acetone.
11. Spread bags out on a wooden or metal tray with an absorbant paper underneath let air dry.
12. Place the bags in an oven and dry at (105 ^{0}C), take the weight (W3)

Determination of lignin and cellulose

1. Place the bags in a 250 ml beaker
2. Add enough sulphuric (technical) to completely cover all the bags.
3. Allow the acid to digest the material for 3 hour while stirring for thorough sample/acid reaction while continuously agitating the content. During the three hours you can change the acid twice or thrice to allow for a complete digestion
4. Place in another beaker containing hot distilled water. Continue and wash until all the acid has been washed away, litmus test can confirm this stage
5. Dry the sample bags in an oven for at least two hours cool in dessicator and weigh (W4).
6. Place each sample bag in pre-weighed © cintered crucible clearly labelled. Place the crucible and the content in an ambient temperature furnace and set the temperature at 500 ^{0}C maintain the temperature for at least 6-8 hours. Remove and cool in dessicator to slightly above room temperature, weigh the crucible and the ash (W5)

Calculations

$$\text{Ash-containing ADF (\%)} = \frac{(W3-W2)}{W1} \times 100$$

$$\text{Lignin (\%)} = \frac{(W3-W4)}{W1} \times 100$$

$$\text{Cellulose (\%)} = \frac{(W4-W5)}{W1} \times 100$$

Remarks. Octyl-alcohol produce the strong smell on heating and the procedure is best done in fume-hood. Acetone is highly flammable. The CTAB/sulphuric solution may require filtering if cloudy. The sample should not be cooled below the room temperature as this absorbs moisture.

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CHAPTER 27

SOLUBLE PLANT PHENOLIC COMPOUNDS

Principle. Polyphenolics are compounds that have a hydroxyl group bonded to an aromatic ring (Palm and Rowland, 1997). Lignin and other plant polyphenolic compounds (Harborne, 1997) resist decomposition and complex with nitrogen in ways that also reduce nutrient availability (Palm *et al*., 2001). In this procedure, polyphenolic compounds in plant tissues are extracted with methanol and total soluble polyphenolics are analysed by the Folin-Denis method (Waterman and Mole, 1994). The analysed polyphenolics comprise the hydrolysable tannins and condensed tannins, as well as non-tannin polyphenolics. Swain (1979) reports extraction from 30% to 95% of total polyphenolics using this method. Constantinides and Fownes (1994) reported less polyphenolics in materials dried at 50^{o}C than in the same material dried at 23^{o}C. Palm and Rowland (1997) advise that samples intended for the analysis of polyphenolic compounds be dried at 35 to 40^{o}C.

Reagents

1. Methanol, 50%
2. Orthophosphoric acid
3. Sodium tungstate
4. Tannic acid
5. Sodium carbonate: Dissolve 17 g sodium carbonate in100-ml distilled water
6. Folin-Denis Reagent: Add 50 g sodium tungstate, 10 g phosphomolybdic acid and 25 ml orthophosphoric acid to 375 ml water and reflux for 2 hours. Cool and dilute to 500 ml with distilled water.

Remark. Glass beads should be added to the flask to avoid superheating.

Standard. Prepare a tannic acid standard at 50 mg litre^{-1} by placing 0.025 g of tannic acid into a 500 ml volumetric flask, dissolve in distilled water and and fill to the 500 ml mark.

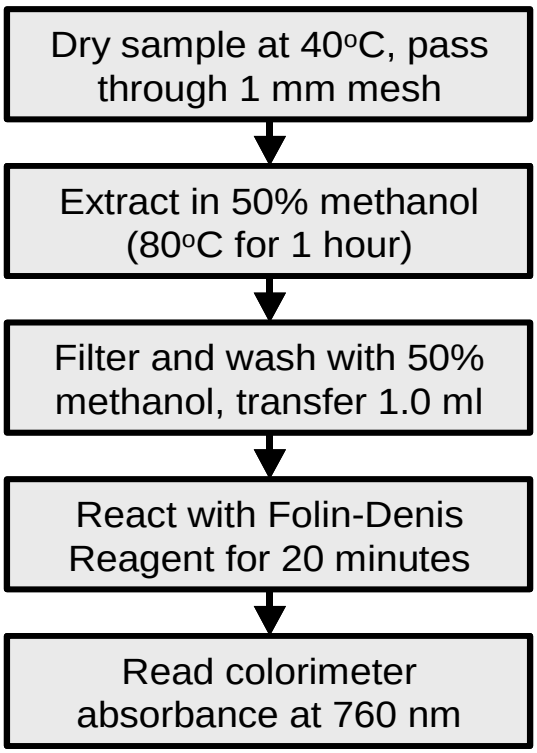

Figure 27.1. Steps in the extraction and measurement of soluble polyphenolic compounds.

Procedure

1. Transfer 0, 1.0, 2.0, 4.0 and 6.0 ml of the tannic acid standard solution into a clean set of five 50 ml volumetric flasks by pipette. This standard series contains 0, 1, 2, 4 and 6 mg tannin litre^{-1} (ppm), respectively.
2. Place 0.01 g (W) of ground plant material dried at 70^{0}C and passed through a 1 mm mesh into a 50 ml cornical flask. Add 20 ml of 50% methanol, cover with a parafilm and place in a water bath at 77-80^{o}C for 1 hour (Figure 27.1).
3. Filter the extract through Whatman filter paper No.1 into a 50 ml volumetric flask, rinse the filter paper with the 50% methanol and fill to the 50 ml mark with distilled water.
4. Pipette 1.0 ml of the standard series and 1.0 ml of the sample solution into a series of 50 ml volumetric flasks. Add 20 ml distilled water, 2.5 ml of Folin-Denis Reagent and 10 ml of 17%

sodium carbonate. Mix well and fill to the 50 ml mark with distilled water. Allow to stand for 20 minutes.

5. Read absobance at wavelength 760 nm using a colorimeter. Plot a calibration curve with the standards and determine the concentration of the samples.

Calculation

$$\textbf{polyphenolics \%} = \frac{\textbf{(a-b)} \times \textbf{v} \times \textbf{100}}{\textbf{w}}$$

where a = concentration of total polyphenolics in the sample extract, b = concentration of the total polyphenolics in the blank extracts, v = total volume of the extract and w = weight of the sample taken

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CHAPTER 28

INTERPRETING SOIL AND PLANT EXPERIMENTAL DATA

Scientists and land managers alike must be aware of both the power and limitations of data collected through the analysis of soils and plants. Single or few measurements have relatively little meaning unless they are viewed within a wider context and compared to the findings of others. These comparisons must be performed with caution as the data sets and inferences of others may have been generated under conditions too dissimilar for direct and non-rigorous application. At the same time, it is understood that land managers are seldom in a position to submit large numbers of soil or plant samples in order to diagnose a simple condition, as with the need for a specific fertilizer formulation. To simplify this dilemma, we suggest that there are two fundamentally different sorts of soil and plant information collected, those that are intended for immediate diagnostic application (usually by land managers) and those that are intended as evidence within a scientific investigation (usually by agricultural and environmental scientists). These two sorts of information are contrasted in Table 28.1

Table 28.1. Differences in soil and plant information collected for diagnosis by land managers and as evidence by scientists.

Intention	Data Set	Analysis	Application
Diagnosis	Few or small	Tabular or heuristic	To direct near-term actions concerning land management.
Investigation	Larger, replicated	Statistical, model Validation	To test hypotheses, answer research questions or validate models

To some extent the separation between diagnostic and evidential data is superficial. Diagnostic information may be compiled over time and among land managers to produce a data set for scientific analysis. Similarly, tables that are constructed to produce recommendations from diagnostic information are the result of previous scientific inquiry. Heuristic knowledge is based upon "experience and common wisdom" and often leads to the same outcomes that a model seeks to capture. Nonetheless, we stand by this practical division of plant and soil information based upon its intended application and direct those who seek more information on diagnostic application of plant and soil analysis to Chapters 29 and 30, respectively. These Chapters contain numerous tables that can be employed in the interpretation of soil or crop information based upon critical concentration thresholds.

Science is the "business" or framing and answering questions about what is not known in order to assist society to address its concerns (Booth *et al*., 1995). Scientists pose hypotheses concerning the relationship between phenomenon, and then design and execute experiments intended to test those hypotheses. Hypotheses should be explicit, with a reader able to determine which treatment combinations and measurements are necessary for testing. This process distinguishes diagnostic and investigative intents. The remainder of this chapter is devoted to scientific approaches to the application of soil and plant data.

Table 28.2 Nutrient Use Efficiency of crops receiving inputs of mineral fertilizer and crop residues (after Sisworo et al., 1990).

crop	rainfall (mm)	------- NUE (%) --------		residue applied
		fertilizer	residue	
upland rice	900 – 1300	9 – 18	11 – 27	cowpea trash
maize – soyabean	410 – 840	42 – 40	2 – 4	rice straw
cowpea	130 – 350	15 – 40	6 – 14	maize/soy stover

Nutrient Use Efficiency. A fundamental issue to improved nutrient management is the utilization efficiency with which plants capture nutrients applied in different forms and placements. Mineral fertilizers are tends to be rapidly solubilized into forms assimilated by plants but at the same time may be lost from soil's root zone by leaching and volatilization. Nutrients applied as organic inputs must first be mineralised through decomposition, but then become subject to the same competing processes of plant uptake and loss from the soil (Myers *et al*., 1994). Regardless of the form, placement or timing of nutrient application, the uptake of nutrients may be assessed through the measurement of Nutrient Use Efficiency (NUE) where:

$$\textbf{NUE (\%) = (nutrient uptake / nutrients applied) x 100}$$

The above equation is somewhat simplified because nutrient uptake must be adjusted for the plant nutrients provided not only by the input, but also those available from the soil in absence of inputs. This adjustment is made by including a control treatment that receives no nutrient inputs, and then subtracting the nutrients contained in the plants of the control from those in the treatments. Since there are no nutrients applied in the control, the equation then becomes:

$$\textbf{NUE (\%)} = [(NU_T - NU_C) / NA_T] \times 100$$

where NU_T is a nutrient measured in plants receiving nutrient input treatments, NU_C is the same nutrient contained in the control without nutrient inputs and NA_T are the total amount of nutrients applied as inputs. In general, nutrients applied as mineral fertilizers have a higher NUE than those applied as organic materials (Table 28.2) and organic materials higher in nutrients tend to have greater NUE than "low-quality inputs due to immobilization and the presence of secondary compounds, particularly lignin and other polyphenols (Myers *et al*., 1994). Higher rainfall tends to lower the NUE of fertilizers due to increased leaching (Sisworo *et al*., 1990).

While the NUE of a given fertilizer or organic resource is useful in predicting crop response to inputs, it must be interpreted with caution. The value obtained tends to be site specific as it is related to moisture, temperature and soil texture. Furthermore, NUE tends to be an overestimate because plants provided external nutrient inputs are better able to extract soil nutrients from a larger volume of soil. Another weakness is that researchers usually confine their samples to above-ground biomass, resulting in a possible underestimate of NUE. Organic inputs generally have low NUE (Table 27.2) but the non-decomposed fraction has additional benefits to the soil. An elegant approach to NUE is captured in the Synchrony Hypothesis which may be paraphrased as *"the release of nutrients from inputs may be synchronized with plant growth demands through the*

quality, placement and timing of organic input application" (Woomer and Swift, 1994). Those with interests in more mechanistic approaches to nutrient dynamics are advised to examine nutrient balances or to apply collected data to the initialisation and validation of computer simulation models.

Nutrient Balances. Sanchez *et al*. (1997) stated *"soil fertility depletion in smallholder farms is the fundamental bio-physical root cause of declining per capita food production in Africa, and soil fertility replenishment should be considered as an investment in natural resource capital"*. To a large extent, the purpose of this book is to better equip African scientists to contribute more information on soil fertility depletion and replenishment, and to allow for stronger comparisons based upon greater standardization of methods. The statement of Sanchez et al. (1997) is largely built upon the studies of Smaling *et al*. (1993, 1997) where nutrient balances were estimated, compared and predicted at different scales. The basis of these estimations may be summarized as:

$$\textbf{Nutrient balance} = [\Sigma\ \textbf{Inputs}_{(1...5)}] - [\Sigma\ \textbf{Outputs}_{(1...5)}]$$

Where Inputs 1 to 5 are IN 1) mineral fertilizer, IN 2) organic inputs, IN 3) atmospheric deposition, IN 4) biological nitrogen fixation and IN 5) sedimentation and Outputs 1 to 5 are the nutrients contained in OUT 1) harvest products, OUT 2) removed crop residues, OUT 3) leaching, OUT 4) volatilisation and OUT 5) runoff/erosion, respectively.

Smaling *et al*. (1993, 1997) developed estimates of the N, P and K balances for different land managements and agroecological zones on a unit area basis (e.g. ha) and then, to develop regional estimates, extrapolated by the coverage (area). An example of this approach appears in Table 28.3. We call this Table to the reader's attention for two reasons, first that the cumulative nutrient depletion within Africa is massive (e.g.4,400,000,000 kg N yr^{-1}) and secondly to note that analytical methods for crops, residues, soils and organic inputs included within this book have an important role within import investigations, even at the continental scale!

Table 28.3. Nutrient depletion rates in cultivated lands in East, Southern and Sub-Saharan Africa (after Smaling *et al*., 1997).

Parameter	East Africa	Southern Africa	Sub-Saharan Africa
Cultivated land ($x10^6$ ha)	39	24	201
Depletion rate (kg $ha^{-1}yr^{-1}$)			
Nitrogen	36	20	22
Phosphorus	5	2	2.5
Potassium	25	14	15
Total depletion (t yr^{-1} x 10^6)			
Nitrogen	1.38	0.48	4.40
Phosphorus	0.19	0.06	0.55
Potassium	0.97	0.32	3.03

Nutrient Need by Expected Yield. Another interesting approach involves the prediction of fertilizer requirement based upon expected yield. A conceptual model to determine the required amount of nitrogen fertilizer in an N-limited soil is:

fertilizer N requirement = uncontrolled N loss + harvested N removal – available soil N + mineralizable soil N – other N credits

or as

$$\textbf{N need} = \textbf{constant} + (\textbf{A} \times \textbf{EY}) - (\textbf{B} \times \textbf{NO}_3\textbf{-N}) - (\textbf{C} \times \textbf{SOM}) - (\textbf{D} \times \textbf{ONC})$$

where the constant represents the sum of leached and runoff/eroded N, A is the N concentration of EY (expected yield), NO_3-N is measured soil nitrate, C is the potential mineralised N in the soil organic matter (SOM) abd D is the mineralization and use efficiency of other residual or added organic sources of N.

This approach is employed in Nebraska (USA) and is described by Shapiro et al. (2001) in an extension manual *"Fertilizer Suggestions for Corn"* (Maize) through an equation that may be converted from pounds and bushels per acre into kg ha^{-1} as:

$$\textbf{N need (kg ha}^{-1}\textbf{)} = 39 + (0.02\ \textbf{EY kg ha}^{-1}) - (4.0\ \textbf{NO}_3) - (0.015\ \textbf{SOM-N kg ha}^{-1})$$

where the N grain content is 2%, each ppm of soil nitrate provides 4 kg available N ha^{-1} and 1.5% of the SOM-N can be expected to mineralise and be available to the crop each season. Note that the constant closely resembles the annual N losses from outputs 3, 4 and 5 in East Africa identified by Smaling *et al*. (1997). Once the multiple regression model is tested, and the coefficients are refined, Nitrogen Need may then be expressed in tabular form for different soil organic matter and nitrate conditions (e.g. Table 28.4).

Table 28.4. An example of a tabular estimate of fertilizer N needs based on expected yield and soil nitrate level for a soil of intermediate soil organic carbon (2%)[a] (after Shapiro *et al*., 2001).

Soil Nitrate Level	Expected Yield (t ha^{-1})						
	2	3	4	5	6	7	8
Very low (1 mg kg^{-1})	27	47	67	87	107	127	147
Low (3 mg kg^{-1})	19	39	59	79	99	119	139
Medium (9 mg kg^{-1})	0	15	35	55	75	95	115
High (15 mg kg^{-1})		0	11	31	51	71	91
Very high (24 mg kg^{-1})			0	0	15	35	55

[a] SOM-N assumes a rooting profile of 20 cm, bulk density of 1.2 kg l^{-1}, a C:N ratio of 15 and that 1.5% of SOM-N mineralises each season,

Initialising and validating computer simulation models. Computer simulation models are becoming an importantly more important research tool in the area of agroecology, particularly because they allow scientists to simultaneously examine the likely effects of land management and climate change on crop production and environmental quality. Examples of such models include CENTURY (Metherell *et al*., 1993; Parton et al., 1994), Rothamsted C (Jenkinson *et al*., 1990) and SOCRATES (Grace and Ladd, 1995). Obtaining the skills and data necessary to initialize and validate complex simulation models are challenging tasks (Table 28.5), but the potential rewards to exploration with a well-calibrated model are great. Models may be used to pre-select among a large number of possible land management treatments, which streamlines field efforts. Also the longer-term impacts of experimental results may be projected over decades. An example of this projection is presented for the simulated total system carbon and its component pools at Kariti in Central Kenya (Figure 28.1) where an upland forest was converted to shifting then permanent agriculture, and now has limited potential to sequester carbon through recommended management practices (Woomer *et al*., 2001). Many of the chapters of this book allow researchers to collect data essential for the initialisation and validation of these computer simulation models (Table 28.5)

Table 28.5. Selected data requirements to initialise the Century model that involve soil and plant analytical data (after Woomer *et al*., 1994).

Model Parameter	Century Model Code	Comments
clay content	CLAY	see Chapter 6
sand content	SAND	see Chapter 6
silt content	SILT	see Chapter 6
soil bulk density	BULKD	see Chapter 7
soil water holding capacity	AFIEL	see Chapter 7
soil pH	PH	see Chapter 8
total soil N	MINERL(1)	see Chapter 9
total soil P	MINERL(2)	see Chapter 9
starting soil microbial C	SOM1C	see Chapter 25
soil microbial C:N and C:P ratios	RCES1	see Chapter 25
starting particulate soil organic C	SOM2C	see Chapter 24
particulate organic C;N and C:P ratios	RCES2	see Chapter 24
starting “humus” C	SOM3C	SOM3C = Total C – SOM1C – SOM2C
“humus” C:N and C:P ratios	RCES3	calculated (above)
crop tissue carbon content	AGLCIS(1)	see Chapter 23
crop tissue nitrogen content	AGLIVE(1)	see Chapter 9
crop tissue phosphorus content	AGLIVE(2)	see Chapter 9
surface litter carbon	STDCIS(1)	see Chapter 23
organic input carbon content	ASTGC	see Chapter 23
organic input nitrogen content	ASTREC1	see Chapter 9
organic input phosphorus content	ASTREC2	see Chapter 9
organic input lignin:C content	ASTLIG	see Chapter 26

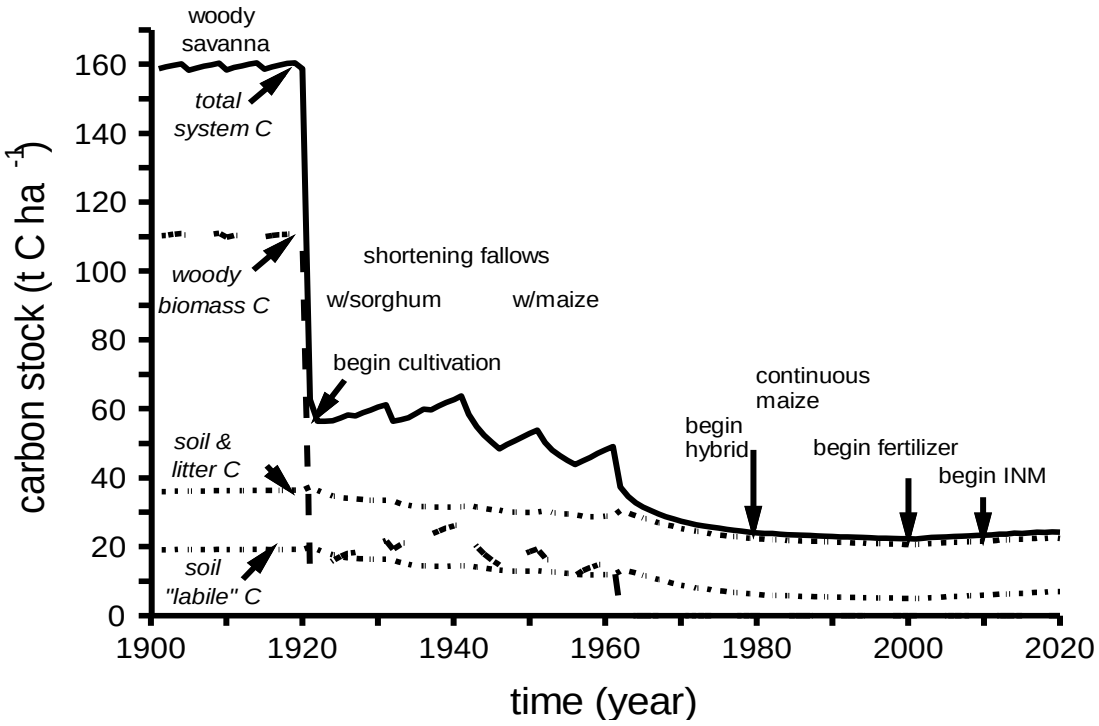

Figure 28.1. Land use change and carbon stocks in Kariti, Central Kenya.

A CENTURY Model simulation (Parton *et al*., 1994) was generated to estimate the changes in various carbon pools resulting from land management. Kariti is located approximately 60 km north of Nairobi in the sub-humid Central Kenyan Highlands, contains loamy clay Nitisol and is densely settled by smallholders practicing maize-based agriculture. First, the simulation was initialized as a lightly-grazed woody montane savanna for 920 years, the last 20 years of which appears on the line graph (1900 to 1920). The woodland equilibrates with approximately 160 t C ha^{-1}, 69% of which is woody biomass and 23% is soil C (Figure 28.1). The woodland is placed into shifting cultivation in 1920 for 40 years, first in sorghum and then maize with shortening fallow intervals. This management reduces system C stocks to about 50-60 t C ha^{-1} with most of the loss occurring from reduced woody biomass. Reduction is soil C (-8 t C ha^{-1}) is largely derived from the "slow" (labile) pool. Continuous maize cultivation commences in 1960 with changes in management reflecting smallholder adoption of hybrid maize (1980) and mineral fertilizers (40 kg N ha^{-1} $crop^{-1}$) from 2000 to 2010 followed by Integrated Nutrient Management (2010 and afterwards). Continuous cultivation results in a further 20 t ha^{-1} decline in total system C as remnant woody biomass removal and labile soil C with almost all system C now contained in the soil. Slight increases in soil C are predicted as a result of adopting Integrated Nutrient Management (INM), a system in which fertilizers are applied to every crop (20 kg N ha^{-1}) and cattle manure (1.5 t ha^{-1}) is applied three out of five years. The carbon stocks resulting from this simulation are in fair agreement with those measured by Woomer et al. (1997) except that the Century Model does not consider soil C at depths >20 cm. Additional improvement in total system carbon would likely require adoption of agroforestry practices. This simulation is based upon a CENTURY Model event file developed by Dr. J.K. Lekasi of Kenya's National Agricultural Research Centre, KARI-Muguga.

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CHAPTER 29

INTERPRETING INFORMATION ABOUT ORGANIC RESOURCES AND CARBON POOLS

Organic resource management has acquired increased attention by agricultural scientists for several reasons. Integrated nutrient management, which seeks to make optimal use of mineral, organic and biological sources of nutrients, is viewed as an important approach for assisting the rural poor and developing more environment-friendly systems of agriculture (Jenssen, 1993; Woomer *et al*., 1999). The organic farming movement, which relies primarily on the recycling of organic resources to supply crops with plant nutrients, is gaining momentum among many grassroots organizations (Rundgren, 1998). Carbon sequestration, seen as an essential mid-term mitigation measure to global climate change, may be achieved in agricultural systems through better use of available organic resources and reduced intensity of cultivation (Lal *et al*., 1998). Each of these approaches rely upon an understanding of the chemical composition of organic resources and their interactions within the soil environment.

Allocation of organic inputs. A novel approach to organic resource management is captured in the decision tree presented in Figure 29.1. This diagram combines two approaches, one based on plant tissue analysis and another on field assessment, that lead to decision making concerning the allocation of available organic resources (Mafongoya *et al*., 1998; Giller, 2000; Palm *et al*., 2001). Built into the decision tree are assumptions concerning nitrogen mineralization, immobilization, priming and polyphenol binding, processes that regulate the availability of nitrogen applied as organic inputs to plants (Cadisch and Giller, 1997). As with any simplified heuristic model, some complications may arise in its application. For example, a green leaf may contain less than 2.5% N (Palm et al., 2001) but still be well suited as a direct input to soil (Woomer *et al*., 1999) or an organic resource may achieve greater value when fed to animals, both in terms of livestock weight gain and manure availability (Lekasi et al., 2001) despite its "depreciation" in organic resource quality. Nonetheless, this decision tree represents an elegant approach to organic resource management and readers are encouraged to apply the methods presented in Chapters 9 (total N), 26 (lignin) and 27 (polyphenols) of this book so that it may be applied to their situations. The chemical compositions of several commonly available organic resources appear in Table 29.1.

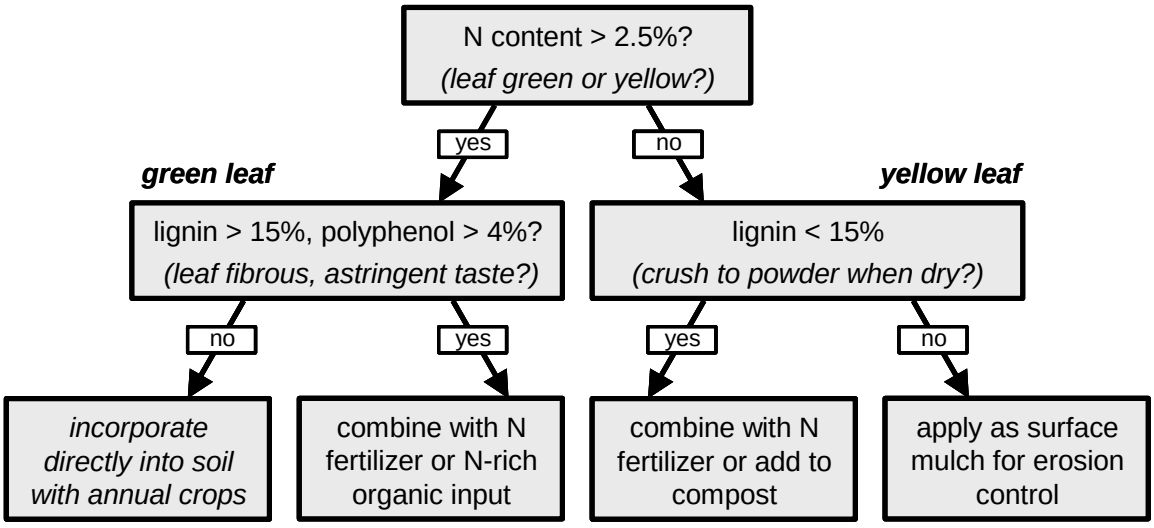

Figure 29.1. A decision tree to guide allocation of available organic resources used as inputs to soil (after Giller *et al*., 2000 and Palm *et a*l., 2001).

Table 29.1. Chemical compositions of selected organic resources available to farmers in East Africa (after Lekasi *et al*., 2001; Palm *et al*., 2001; Woomer *et al*., 1999).

organic resource	N	P	K	lignin	polyphenol
	---------- % of dried material ----------				
fresh wood chips for poultry bedding	0.43	0.04	0.12	high	--
cowpea trash (*Vigna unguiculata*)	0.57	0.05	1.79	14.7	1.30
banana trash (*Musa* spp.)	0.83	0.06	4.54	low	--
maize stover (*Zea mays*)	0.89	0.08	2.78	4.90	1.46
coffee husks (*Coffee arabica*)	1.01	0.05	1.06	--	--
napier grass (*Pennisetum purpureum*)	1.02	0.11	2.63	4.82	0.95
bean trash (*Phaseolus vulgaris*)	1.20	0.13	2.06	21.20	--
Lablab purpureus cuttings	1.31	0.33	--	8.00	2.80
pigeon pea prunings (*Cajanus cajan*)	1.33	0.10	1.01	8.10	3.10
sweet potato vines (*Ipomoea batatas*)	2.27	0.14	3.05	low	--
Tephrosia vogelii prunings	2.47	--	--	9.28	4.92
Calliandra calothryrsu prunings	2.56	--	--	12.33	13.23
Tithonia diversifolia prunings	2.57	--	--	11.96	3.43
Gliricidia sepium prunings	3.43	0.26	--	15.50	1.20
Desmodium uncinatum cuttings	3.44	--	--	9.43	3.12
young soyabean leaves (*Glycine max*)	3.67	--	--	7.24	1.01
Leucaena leucocephala prunings	3.91	--	--	17.56	1.64

Manure and compost management. A key organic resource available to smallhold farmers is livestock manure and composts produced from combinations of faeces, urine and feed refusal (Lekasi *et al*., 2001). Compost production is an important farm activity because it allows for organic resources to be accumulated until they are needed prior to the planting season although nutrients may be lost if materials are improperly handled or stored. The nutrient contents of manures and composts collected from visits to 190 smallhold farms in the central highlands of Kenya is presented in Table 29.2. Conditions under which cattle are reared may also affect the quality of manure that is prepared from animal stalls (Table 29.3). Readers should note that the maize yields obtained from incorporating manure-based composts (Table 29.3) as a pre-plant application at a rate of 75 kg N ha^{-1} result in substantial yield improvement (Table 29.4), an effect that would not be predicted from the decision tree based upon plant material (Figure 29.1).

Table 29.2. The use of manures and composts as soil inputs among smallholders in the central Kenyan highlands and their nutrient contents (from Woomer *et a*l., 1999).

Resource	household use as soil input	minerals (ash)	---- nutrient content ---- N	P	K
	----------	----------	---------- % ----------	----------	----------
Cattle boma manure	92	53	1.40	0.20	2.38
Poultry manure	65	35	3.11	0.42	2.40
Goat/ sheep manure	65	41	1.48	0.20	3.31
Domestic compost	58	70	1.34	0.20	1.82
Swine manure	6	63	1.40	0.23	2.02

Table 29.3. Chemical compositions of composts prepared from materials collected under different livestock rearing conditions (from Lekasi *et al*., 2001).

Composted material[1]	Faeces (F)	F& urine (U)	F& refusal (R)	F& R& U
Total Kjeldhal nitrogen (%)	1.59	1.60	1.91	1.76
Total phosphorus (%)	0.61	0.64	0.58	0.54
Organic carbon (%)	36.8	35.9	34.8	35.4
Lignin (%)	21.6	17.2	27.1	27.3
Polyphenols (%)	0.84	1.35	0.88	1.40

[1] refusal is the feed dropped on the stall floor by cattle and F&U&R consists of faeces, urine and feed refusals mixed on the stall floor by the cattle.

Farmers in East Africa rely on observation to assess the quality of composts based upon a compost's texture, color, age, smell and the presence of invertebrates. Reliance upon these criteria allow farmers to distinguish composts with favorable chemical characteristics (Lekasi *et al*., 2001). Composts that are older than five months with dark color and uniform, fine texture contain higher levels of nitrogen and phosphorus than those that were younger with lighter color and heterogeneous texture. Smell and the presence of invertebrates are less accurately employed as farmer criteria of compost quality, arguably because finished is nearly odorless and may be considered more "substrate-depleted" for heterothrophic organisms. The relative reliability of farmer's criteria toward resource quality agree with studies elsewhere by Motavalli *et al*. (1994) and Garforth and Gregory (1997), and reinforces the role of participatory approaches and indigenous knowledge within activities aimed at on-farm problem solving (Chambers *et al*., 1990).

Carbon sequestration in soils. The role of soil management in climate change is becoming more widely appreciated among the scientific community. Briefly, atmospheric carbon dioxide has increased from the pre-industrial level of 275 ppm to a current 350 ppm. This increase has resulted in increased temperatures and changing weather patterns that will have a profound impact on

Table 29.4. Maize yields resulting from the addition of different composted manures over two growing seasons at Kariti, Central Kenya (from Lekasi *et al*., 2001).

Input[1]	--- input rate ---		--- maize grain yield ---	
	N	P	first season	second season
	---------- kg ha^{-1} ----------			
none	0	0	1371	971
composted F & urine (U)	75	30	2916	1142
composted faeces (F)	75	29	3592	1402
composted F & U & FR	75	23	3996	1542
composted F & feed refusals (FR)	75	23	4336	1564
$LSD_{0.05}$			1140	505

[1] chemical compositions of the composts are described in Table 29.3.

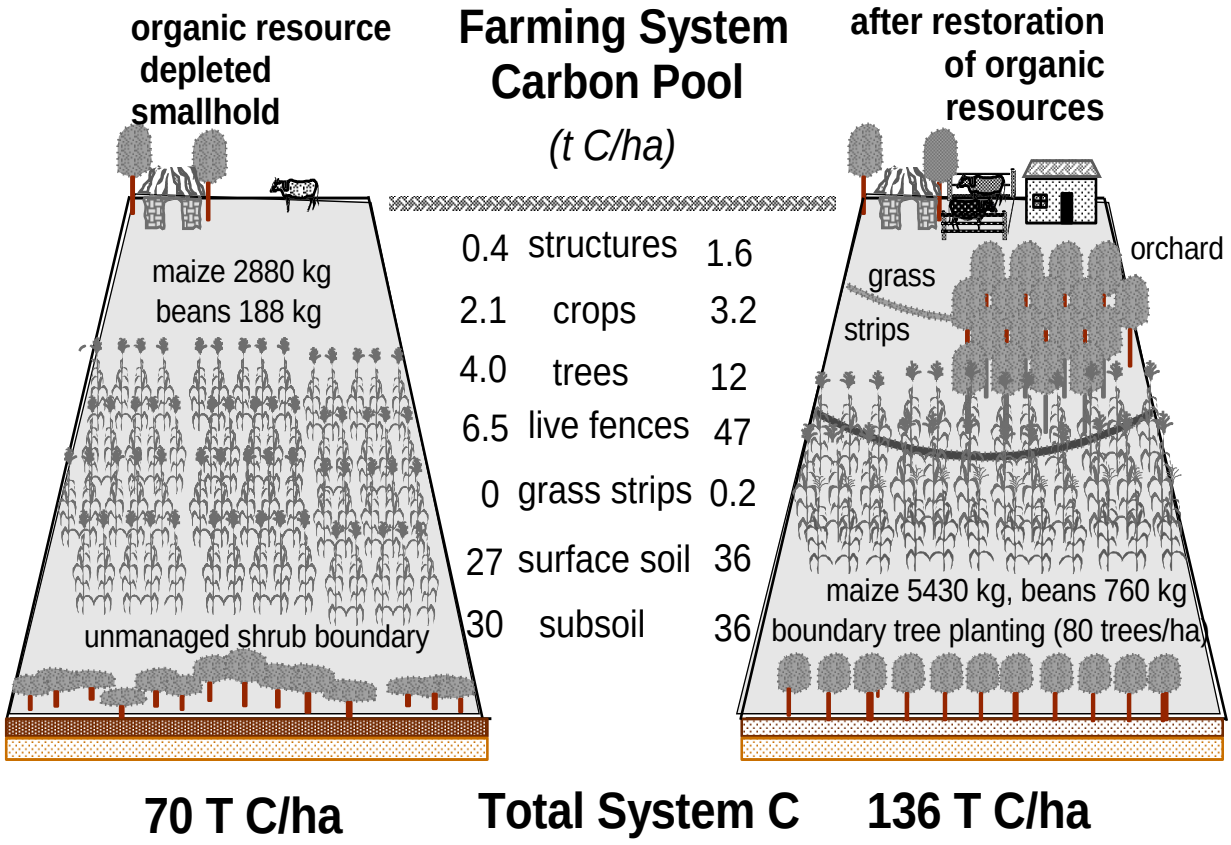

Figure 29.1. Potential exists to re-accumulate carbon within smallhold farming systems in Africa (from Woomer *et al*., 1997).

human society and the earth's natural environment unless ameliorative actions (known as mitigation) are taken. Land use change and soil management are the second leading cause of atmospheric change, following the combustion of fossil fuels. Large areas of forests were cleared for agriculture over the past centuries and the biomass carbon lost to the atmosphere. Intensive cultivation further reduces the carbon stocks in agricultural land (Post and Mann, 1990) to the extent that conservation tillage offers tremendous potential to re-accumulate (sequester) carbon in temperate agriculture (Lal *et al*., 1998).

Land management practices that result in the re-accumulation of carbon (known as sequestration) as biomass and soil organic matter often have a win-win impact within smallhold farming systems as they tend to become more productive and to offer greater global environmental services. Woomer *et al.* (1997) estimated that a step-wise re-accumulation of farming system carbon involving tree planting and soil conservation in the east African highlands has the potential to increase carbon stocks from 70 to over 130 t C ha-1 and to increase crop yields by about 40% (Figure 29.1). Additional information on the role of tropical soils to mitigate greenhouse gas emissions may be obtained from Bouwman (1990), Wisniewski and Sampson (1993) and Lal *et al.* (1998, 2000).

Estimating the carbon stocks in mixed-enterprise farming systems requires that carbon measurements of woody and herbaceous biomass, litter, roots, tilled soil layer and subsoils be performed, and these measurements be compiled in a manner that reflects their proportion within the farming system. Woody biomass is estimated using allometric approaches (FAO, 1997) while herbaceous biomass is collected, dried and weighed (Woomer and Palm, 1998). The carbon content of biomass and intact litter may be inferred from mass (e.g. 0.45 to 0.50) but the C contents of soils and partially-decomposed litter require chemical analysis or combustion.

Root measurement is a necessary component of detailed investigations comparing candidate management interventions but is too time consuming for purposes of routine monitoring. Roots are collected by excavating a known soil volume with a narrow, flat-bladed shovel and hand saw. Coarse roots are hand-sorted and washed. The remaining sample is dispersed in tap water, passed through a 2 mm sieve and roots collected without attempt to differentiate live and dead roots. Roots are then washed of gross mineral contamination, dried at 65^{o} to constant weight, weighed and a sub-sample ground and ashed. Ash-corrected dry weight (Anderson and Ingram, 1993) is assumed to contain 0.45% carbon. Soils are recovered to the desired depth using a narrow, flat-bladed shovel and the soils are measured for total organic carbon (Chapter 23) or any other soil carbon fraction of interest (e.g. Chapters 24 and 25). Soil bulk density must also be measured to calculate total carbon on an area basis (Lal and Kimble, 2001; see Chapter 7).

After all carbon stocks are converted into the same dimensions (e.g. t C ha^{-1}), total system carbon is calculated as:

$$\mathbf{C_{total} = C_{agb} + C_{root} + C_{litter} + C_{soil}}$$

where C_{total} = total system C on an area basis, Cagb = carbon in the aboveground woody and herbaceous biomass, C_{root} = the carbon in the roots and below-ground litter and C_{soil} = the total soil organic matter. To calculate total system carbon stocks, the individual pools within each farm enterprise: woody biomass, herbaceous biomass, litter, roots and soil, are totalled and expressed as Mg carbon ha^{-1} (Woomer and Palm, 1998; Woomer *et al*., 2001). Next, the total area of each enterprise, expressed in hectares (ha), is multiplied by their respective stocks and then farm enterprises are totalled. Different farms, not field replicates within farms or individual quadrates, must be regarded as statistical replicates when estimates of error are produced. More detailed information may be obtained on the measurement of total system carbon and its component pools, in natural and managed ecosystems from Anderson and Ingram (1993), Woomer and Palm (1998), Woomer *et al.* (2001) and Lal *et al.* (2001). Soil scientists are well advised to familiarize themselves with the measurement of system carbon pools and fluxes, and the potential role of soil management in the mitigation of climate change as these are issues that will become increasingly important over the next several decades.

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CHAPTER 30

INTERPRETING PLANT ANALYSIS DATA BY CRITICAL NUTRIENT CONCENTRATION

Principle. Different approaches have been used worldwide for interpreting plant analytical results. These include empirical studies related to plant response to a specific nutrient, nutrient uptake, nutrient survey, nutrient ratios, spot tests, biochemical and physiological tests. These approaches are adequately documented and explained in standard textbooks on plant nutrition and interpretation manuals (see Reuter and Robinson, 1986).

Critical Nutrient Concentration. The concept of critical nutrient concentrations forms the basis of most methods for using plant analysis for assessing plant nutrient status. Ulrich (1952) proposed three definitions of the critical nutrient concentration:

- The nutrient concentration that is just adequate for maximum growth
- The concentration separating the zone of deficiency from the zone of adequacy
- The concentration that is just deficient for maximum growth

Thus, a single definition of the critical nutrient concentration would not suffice, reflecting a lack of knowledge regarding the functions of elements and lack of a means of measuring the effective concentration of nutrients at sites of reaction within plant cells. Work by Nable and Loneragan (1984a,b) on manganese demonstrates the value of such approach. If such knowledge and technology were available, then it might be possible to define critical nutrient concentration in terms of nutrient concentrations associated with particular physiological reaction limiting growth when the plant material was sampled.

In giving the above definitions of the critical nutrient concentration, Ulrich (1952) pointed out that this usually did not cause problems in practice. The reason, determined experimentally, is that the critical concentration is not a single value but a narrow range of nutrient concentrations, above which the plant is adequately supplied with nutrient, and below which the plant is deficient. Such a range would, therefore, cover the different critical values derived by strict application of different definitions. This view of the critical concentration being a range rather than a single value is often overlooked when critical concentrations are reported or used to interpret plant/soil analyses. Too often, critical concentrations are regarded as single values and rigid boundaries between deficient plants and those adequately supplied with nutrients. A careful study of the data and methodology used to derive a critical nutrient concentration will reveal how inappropriate it is to attempt to use critical concentrations in this way. The effects of the numerous physical, environmental and biological factors (such as genotype differences and tissue age) that influence nutrient levels in plant tissues is a further argument for regarding critical nutrient concentrations as ranges rather than as single values. One means of highlighting the fact that a critical concentration is a range is to provide estimates of errors involved in its derivation.

In conclusion, researchers are encouraged to undertake studies with the aim of providing a better understanding of the transport of nutrients throughout the plant, how these transport processes are regulated, and what effects nutrient stress has on them. It is hoped that such studies will provide information required to develop the sound physiological basis that is needed for more reliable means of interpreting plant analyses and assessing plant nutrient status.

The critical nutrient concentrations for several crops of importance in East Africa are given in this section. The values should be used only as guidelines. It is recognised that most soil/plant tissue testing laboratories have their own values.

Tables of Plant Tissue Nutrient Concentrations. The following tables (Tables 30.1 to 30.7)provide insight into the nutrient concentrations of different plant tissues for several important food and commercial crops in East Africa, banana (*Musa spp.*), beans (*Phaseolis vulgaris*), cabbage (*Brassica oleraceae*). Coffee (*Coffee arabica*), maize (*Zea maize*) and sugarcane (*Saccharum* spp.), respectively. Table 30.7 provides information on P-deficient and P-sufficient maize crops in several Kenyan soils.

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Table 30.1. Nutrient concentrations in plant tissue of banana (*Musa* spp.).

Plant	Concentration Range				
Nutrient	Deficient	Marginal	Critical	Adequate	High
N %	< 2.6	2.6 - 2.8	2.6	2.8 - 4.0	-
P %	< 0.13	0.13 - 0.19	0.2	0.2 - 0.25	> 0.25
K %	< 2.5	2.5 - 3.0	3.0	3.1 - 4.0	> 4.0
N to K	-	-	-	1:1.0 - 1:1.1	-
S %	< 0.1	0.1 -0.2	0.23	0.23 - 0.27	> 0.27
Ca %	< 0.5	0.5 - 0.7	0.5	0.8 - 1.2	> 1.25
Mg %	< 0.20	0.2 - 0.3	0.3	0.3 - 0.46	> 0.46
Na %	-	-	0.005	0.01 - 0.10	-
Cl %	-	-	0.6	0.8 - 0.9	-
Cu mg kg^{-1}	-	3 - 7	9	7 - 20	-
Zn mg kg^{-1}	< 14	14 - 20	18	21 - 35	> 35
Mn mg kg^{-1}	< 10	-	25	100 - 2200	4000 - 6000
Fe mg kg^{-1}	-	-	80	70 - 200	-
B mg kg^{-1}	< 10	10 - 20	11	20 - 80	80 - 300
Mo mg kg^{-1}	-	-	1.5 - 3.2	-	-
Al mg kg^{-1}	-	-	-	50 - 240	-

After Reuter and Robinson (1986). Plant sampling: Growth stage during periods of active growth from medium-sized suckers with broad leaves. Weather conditions may influence the appropriate time of year. Plant part: Strips of 15-20 cm wide from each side of the midrib of the third youngest leaf.

Table 30.2. Nutrient concentrations in plant tissues of bean (*Phaseolus vulgaris*)

Nutrient	Growth Stage	Plant Part	Concentration Range			
			Deficient	Critical	Adequate	Toxic
N %	In bud	TML	-	3.5	4.0 - 6.0	-
	Early flower	TMB	< 5.0	-	5.2 - 5.4	-
	Peak harvest	Pods	-	-	3.1	-
	Seed harvest	Seeds	-	-	4.5	-
P %	In bud	TML	-	0.30	0.32 - 0.50	-
	Early flower	TMB	< 0.25	-	0.4 - 0.6	-
	Peak harvest	Leaflets	-	-	0.24	-
	Peak harvest	Pods	-	-	0.30	-
	Seed harvest	Seeds	-	-	0.36	-
K %	Mid-growth	TMP	3	-	5	-
	In bud	TML	-	1.5	1.8 - 2.5	-
	Early flower	TMB	< 1.2	-	1.5 - 3.5	-
	Peak harvest	Leaflets	-	-	1.9	-
	Peak harvest	Pods	-	-	2.6	-
	Peak harvest	Seeds	-	-	1.3	-
S %	Early growth	WS (dried)	-	-	0.16 - 0.64	1.12
	Peak harvest	Pods	-	-	0.17	-
	Peak harvest	Seeds	-	-	0.22	-
Ca %	In bud	TML	-	-	0.8 - 3.0	-
	Early flower	TMB	1.2	-	1.5 - 2.5	-
	Peak harvest	Leaflets	-	-	2.92	-
	Peak harvest	Pods	-	-	0.52	-
	Peak harvest	Seeds	-	-	0.15	-
Mg %	In bud	TML	-	0.20	0.25 - 0.70	-
	Early flower	TMB	< 0.25	-	0.40 - 0.80	-
	Peak harvest	Leaflets	-	-	0.52	-
	Peak harvest	Pods	-	-	0.24	-
	Seed harvest	Seeds	-	-	0.16	-
Mo mg kg^{-1}	56 DAS	WS	-	-	0.4	-

After Reuter and Robinson (1986). TMB = youngest, uppermost (top) fully mature leaf blade, often the fourth leaf down from the apex of immature plants. TMP = petiole of the TM leaf. TML = blade and petiole of the TM leaf. WS = whole sample (all tops). DAS = days after sowing.

Table 30.3. Nutrient concentrations in plant tissues of cabbage (*Brassica oleracea* var. *capitata*)

	Growth Stage	Plant Part	Deficient	Critical	Adequate	High	Toxic
N (%)	Head	WL	<2.5	2.5	2.5-4.0	-	-
NO_3-N (%)	Head	HT	0.50	-	0.50-1.00	-	-
P %	Head	WL	<0.20	-	0.30-0.50	-	-
K %	Head	WL	<2.00	-	-	2.0-4.0	-
Ca %	Head	WL	<0.50	1.0	2.0-3.0	-	-
Mg %	Head	WL	<0.10	-	0.20-0.60	-	-
Na %	Head	WL	-	-	<1.0	-	-
Cl %	Head	WL	-	-	<2.0	-	-
Cu mg kg^{-1}	55 DAT	WL	-	-	-	-	56-116
	Head	WL	-	-	5.20	-	-
Zn mg kg^{-1}	55 DAT	WL	-	-	-	-	358-805
	Head	WL	<10	-	10-200	-	-
Fe mg kg^{-1}	55 DAT	WL	-	-	-	129	143
	Head	WL	<50	-	50-200	-	-
B mg kg^{-1}	Head	WL	<20	-	30-60	-	-
Mo mg kg^{-1}	Head	WL	<0.1	0.2	0.3-0.5	-	-
Co mg kg^{-1}	55 DAT	WL	-	-	-	-	138-1250

After Reuter and Robinson (1986). DAT= days after transplanting, WL = wrapper leaf, HT = heart core.

Table 30.4. Nutrient concentrations in plant tissues of coffee (*Coffee arabica*).

Plant	Concentration Range				
Nutrient	Deficient	Marginal	Adequate	High	Toxic
N %	<2.2	2.2-2.5	2.5-3.0	>3.0	-
P %	<0.1	0.1-0.15	0.15-0.20	>0.20	-
K %	<1.5	1.5-2.1	2.1-2.6	>2.6	-
SO_4-S %	-	-	0.02-0.1	-	-
Ca %	<0.4	0.4-0.75	0.75-1.5	>1.5	-
Mg %	<0.1	0.1-0.25	0.25-0.40	>0.40	-
Cu mg kg^{-1}	<10	10-16	16-20	>20	-
Zn mg kg^{-1}	<10	10-15	15-30	>30	-
Mn mg kg^{-1}	<25	25-50	50-100	100-700	>700
Fe mg kg^{-1}	<40	40-70	70-200	>200	-
B mg g^{-1}	<25	25-40	40-100	100-200	>200

After Reuter and Robinson (1986). Plant sampling: Fourth pair of leaves from the top of actively growing and bearing branches but not including the terminal leaf pair if less than 5 cm long . Avoid leaves with insect damage. Take four pairs of leaves from each of 20 or 25 trees, midway between ground level and the topmost branches. Keep samples cool and wash in dilute acetic acid (28 ml in 5 l. water) for 10 minutes; drain and rinse in distilled water.

Table 30.5. Nutrient concentrations in plant tissues of Maize (*Zea mays*)

Plant Nutrient	Growth Stage	Plant Part	Concentration Range			
			Deficient	Critical	Adequate	Toxic
N (%)	30 - 45 DAE	WS	-	-	3.5 - 5.0	-
	Full tassel	BOBC	-	3.0	-	-
	Silking	BOBC	-	2.7 - 2.9	-	-
	Silking	Ear leaf blade	< 2.45	-	2.76 - 3.5	> 3.5
P (%)	≤ 30 cm tall	WS	-	-	0.3 - 0.5	-
	30 - 45 DAE	WS	-	-	0.4 - 0.8	-
	Full tassel	BOBC	-	0.25	-	-
	Silking	BOBC	0.22 - 0.32	-	0.27 - 0.62	-
	Silking	Ear leaf blade	< 0.15	-	0.25 - 0.4	> 0.5
K (%)	≤ 30 cm tall	WS	-	-	2.5 - 4.0	-
	30 - 45 DAE	WS	-	-	3.0 - 5.0	-
	Full tassel	BOBC	-	-	1.8 - 3.0	-
	Silking	BOBC	1.5 - 2.7	-	2.1 - 3.0	-
	Silking	Ear leaf blade	< 1.25	-	1.71 - 2.25	> 2.5
S (%)	30 - 45 DAE	WS	-	-	0.2 - 0.3	-
	Full tassel	BOBC	-	0.15	-	-
	Silking	BOBC	-	0.24	-	-
Ca (%)	≤ 30 cm tall	WS	-	-	0.3 - 0.7	-
	30 - 45 DAE	WS	-	-	0.9 - 1.6	-
	Full tassel	Ear leaf blade	-	0.4	-	-
	Silking	Ear leaf blade	< 0.1	-	0.21 - 0.5	-
Mg (%)	≤ 30 cm tall	WS	-	-	0.15 - 0.45	-
	30 - 45 DAE	WS	-	-	0.3 - 0.8	-
	Full tassel	BOBC	< 0.12	-	-	-
	Full tassel	Ear leaf blade	-	0.25	-	-
	Silking	Ear leaf blade	< 0.1	-	0.13 - 0.24	-

After Reuter and Robinson (1986). WS = whole sample (plant tops). BOBC = blade opposite and below cob. BOAC = blade opposite and above cob. DAE = days after emergence.

Table 30.6. Nutrient concentrations in plant tissues of sugarcane (*Saccharum* spp.)

Nutrient	Growth Stage	Plant Part	Concentration range			
			Deficient	Critical	Adequate	Toxic
N %	10 month P	TFDL	<1.7	1.6-2.0	1.9-2.5	-
	7 month R	TFDL	<1.7	1.6-2.0	1.9-2.5	-
	3 month	leaves	<1.4	-	1.6-2.0	-
P %	10 month P	TFDL	<0.18	0.15-0.23	0.21-0.30	-
	7 month R	TFDL	<0.18	0.15-0.23	0.21-0.30	-
	3 month	leaves	<0.15	-	0.18-0.24	-
K %	10 month P	TFDL	<1.1	0.9-1.3	1.3-2.0	-
	7 month R	TFDL	<1.1	0.9-1.3	1.3-2.0	-
	3 month	leaves	<1.4	-	1.55-2.00	-
S %	35 DAS	WS	-	0.36	-	-
	35 DAS	Blades 3-6	-	0.24	-	-
	70 DAS	Blades 3-6	-	0.10	-	-
	70 DAS	Sheaths 3-6	-	0.08	-	-
	18 month	Sheaths 3-6	0.72	-	-	-
Ca %	3 month P	TFDL	<0.15	-	0.2-0.5	-
Mg %	3 month P	TFDL	-	-	0.09-0.12	-
	5-7 month P	TFDL	-	-	0.08	-
Na %	Rapid growth	TFDL	-	-	-	0.04
Cl %	Rapid growth	TFDL	-	-	<0.5	0.6-1.0

After Reuter and Robinson (1986). P = plant crop. R = ratoon crop. TFDL = top fully developed leaf (blade). Sampling is recommended during the 'boom' phase of growth, i.e. when stalk elongation is >2 cm day^{-1}. TFDL = top fully developed leaf, usually the third leaf from the shoot apex. Sample consists of a section, 20 cm long 910 cm above and 10 cm below the midpoint of the FDL blade is taken for chemical analysis. The midrib is discarded.

Table 30.7. A guide to P tissue concentration of maize under P limited and non-limiting soil conditions based on Kenyan studies of P nutrition.

Location	Classification (FAO/UNESCO)	---- soil P ---- Bray 2	Olsen	Shoot P 5-7 wk	Ear leaf at silking	Grain	Stover	P Response[1]
		---- mg kg^{-1} ----		---------- P % ----------				%
Locations where P responses are observed but no P is applied (control plots)								
Kakamega	Eutric Nitisol	11	5	0.17	0.22	0.17	0.05	282
Makueni	Acri-rhodic Ferralsol	9	2	0.16	0.10	0.10	0.05	207
Kitui	Ferral-chromic Luvisol	38	7	0.25	0.19	0.19	0.04	92
Wamunyu	Haplic Alisol	7	5	0.28	--	0.26	0.09	725
Mutua	Haplic Lixisol	8	5	0.23	--	0.13	0.05	421
Kidetok	Orthic Ferralsol	49	4	0.17	0.22	0.26	0.08	86
mean (standard deviation)		*20 (18)*	*5 (2)*	*0.21 (.05)*	*0.18 (.06)*	*0.19 (.06)*	*0.06 (.02)*	*302 (242)*
Sites without P response where no P is applied								
Muguga	Humic Nitisol	55	28	0.49	0.25	0.25	0.08	-11
Kitale	Orthic Ferralsol	40	11	0.42	0.25	0.31	0.13	-21
Kabati	Acri-rhodic Ferralsol	337	74	0.49	0.32	0.32	0.13	12
Kiboko	Orthic Luvisol	108	62	0.29	0.23	0.37	0.12	2
mean (standard deviation)		*135 (138)*	*44 (29)*	*0.42 (.09)*	*0.26 (.04)*	*0.31 (.05)*	*0.12 (.02)*	*-5 (14)*

[1] P Response = [(yield from 60 kg N + 20 kg P/ha treatment - yield from control) / yield from control] x 100

CHAPTER 31

INTERPRETING SOIL ANALYSIS DATA

Principle. One of the objectives of soil testing is to assess the overall nutrient status of soils by using the soil test data as guidelines to provide fertilizer/manure/crop residue recommendations. We must remember that extractable nutrient methodologies are empirically-based approximations of the potential nutrient supply available from the soil to a plant since mechanistic factors, such as the buffer power, which controls the soil solution concentration of nutrients, or the total surface area of active roots are not being considered. As such, the soil test results must be interpreted with some caution. For example, in a study conducted in East Africa, Osborne (1974) obtained no significant correlations between crop response parameters and soil test data. He attributed these results to the considerable variation in soil properties such as the parent material, weathering status and biological properties.

Interpreting C and N data. Although high yields are generally associated with high organic matter content in low external input agriculture, the carbon and nitrogen components of the organic matter are difficult to interpret. The levels of N, in the plant-available NH_4 and NO_3 forms fluctuate during the cropping season and are dependent on factors such as soil moisture (rainfall pattern), cropping history, litter inputs and microbial activity. Nevertheless, Takalign *et al.* (1991) attempted to provide general guidelines regarding the organic carbon and total nitrogen levels in agricultural soils (Table 31.1). More detailed information of the crop response to fertilisers and the associated soil test values is presented in Table 31.2.

While soil organic carbon is not a requirement for plant growth, the levels of organic matter in soils influence a number of soil chemical and physical processes. Soil organic matter affects soil aggregation by binding individual clay particles together into microaggregates and by clustering these into macro-aggregates. Well-aggregated soils demonstrate improved drainage. Soil organic matter improves soil moisture holding and cation exchange capacities. In low external input cropping systems, the mineralisation of organic matter contributes to soil fertility. For all of these reasons, the soil organic matter status is an important indicator of the soil as a rooting environment and the decline in soil organic matter in cultivated soils, when compared to similar, adjacent soils covered with forest, savanna, grassland or mature fallow is an effective measure of the extent of chemical and physical soil degradation.

The capacity of a soil to sequester C is under the control of soil texture. Clay particles physically protect recent organic additions to soils and form stabilised organo-mineral complexes with the humus fraction. On the other hand, sandy soils are well aerated, and their low, non-reactive surface areas are not able to stabilise organic matter to the same extent as clays or loams. Similarly, the recalcitrance (chemical stability) of the organic matter which forms within a soil is also under the control of the soil moisture regime. Generally, the more humid the environment, the greater the proportion of recalcitrant organic matter. The relationship between soil texture, precipitation and the relative loss of soil organic matter that can be expected to result from the conversion of natural vegetation to agriculture is presented in Figure 31.1. When interpreting Figure 31.1, the reader should be aware that the z axis is not a measure of soil organic C but rather the proportion of C that is lost from a soil system when converted from a natural to a managed ecosystem and represents the combined effects of soil disturbance and the decline in soil organic

Table 31.1. General guidelines on the interpretation of soil N and C test results (Tekalign, 1991).

	Measured value	Rating
Organic C (%)	> 3.0	High
	1.5-3.0	Moderate
	0.5-1.5	Low
	< 0.5	Very low
Total N (%)	> 0.25	High
	0.12-0.25	Moderate
	0.05-0.12	Low
	< 0.05	Very low

inputs. In soils low in clay and receiving small amounts of annual precipitation, less soil organic C is lost because the capacity of the original soil system to sequester C is reduced due to ready soil aeration and the reduced physical protection of organic matter by clays. When clayey soils in similar climates are disturbed, the disruption of soil aggregates results in greater exposure of previously protected soil organic C (see Chapter 24) and a greater proportionate decline. In extremely humid environments, the soil organic matter present in the original soil is inherently recalcitrant due to the weathering effects under which it formed and less suseptible to loss upon soil disturbance. Although in extremely humid environments, greater leaching of particulate and dissolved organics occurs. The rate of loss of soil organic C from agroecosystems is also under the control of the organic input management and is mitigated by such practices as retention of crop residues, manuring and conservation tillage.

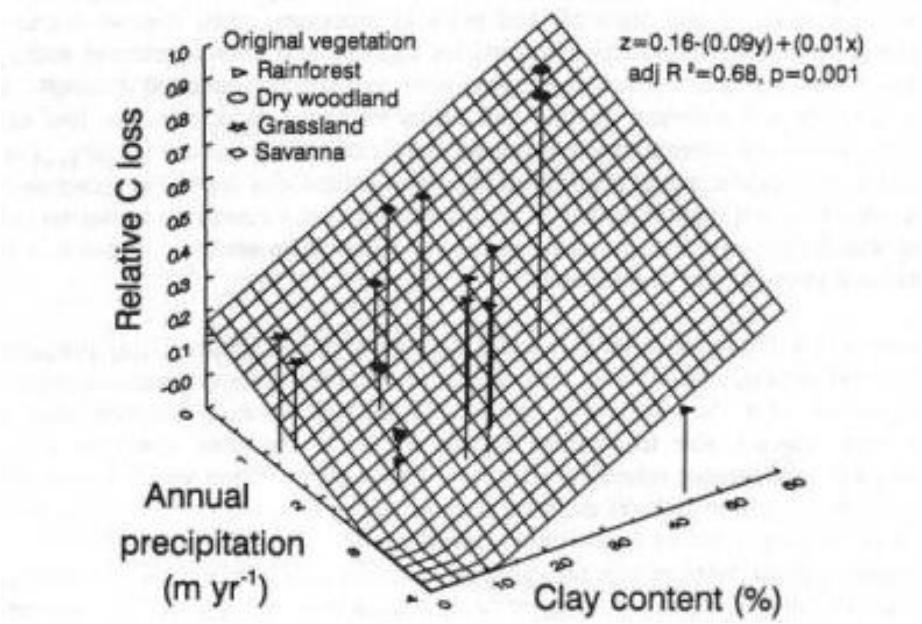

Figure 31.1. The relative decline in soil organic carbon resulting from conversion of natural ecosystems to more intensive land use (after Woomer *et al*., 1994).

Table 31.2. Comparisons of total soil carbon and selected carbon fractions in the Kikuyu Red Clay, a Humic Nitisol, of Central Kenya (after Kapkiyai *et al*., 1998[1] and Murage *et al.*, 2000[2]).

Location (Management)	Microbial C	Particulate C	Total C
	------- mg kg^{-1} -------		(g kg^{-1})
Kabete Long-term Experiment[1]			
No inputs	54	713	13.9
Fertilizer applied (F)	53	695	14.0
Stover retained (S)	72	748	14.9
Manure applied (M)	90	1459	15.4
FM applied, S retained	119	1613	16.9
Twelve nearby Smallhold Farms[2]			
Non-productive land	103	801	19.2
Productive land	145	1236	24.1

[1] after 18 years of management as a maize-bean rotation at the KARI Centre in Kabete, Kenya. F = 123 kg N and 23 kg P ha^{-1} yr^{-1}. M = 10 t ha^{-1} yr^{-1}. [2] based upon interviews at 12 farms in Kiambu District, Kenya and subsequent analyses of soils.

The relative sizes of microbial biomass C, particulate C and total soil C pools, and their response to management in two related studies in the Kikuyu Red Clay (a Nitisol) of Central Kenya are presented in Table 31.2. The methods employed in the studies of Kapkiyai *et al*. (1998) and Murage *et al*. (2000) are similar to those presented in Chapters 23, 24, and 25 of this book. Microbial Biomass C and Particulate Organic C are relatively small components of total soil C, but are much more responsive to soil management history, a phenomenon that lends to their use as indicators of soil quality in a wider range of agroecological zones (Swift and Woomer, 1993).

Interpreting P data. With regard to extractable phosphorus, crop response to phosphate fertilizer has been observed in soils where P test levels are below 10 mg P kg^{-1} soil (10 ppm P), when the routine Bray No.2, Olsen and Truog extractants are used (Roche *et al.*, 1980; Okalebo, 1987; Okalebo *et al.*, 1989). In general, different chemical extractants yield different values of extractable P. The values of P obtained from different methods may be correlated with crop yields, but the relative advantages of different methods are to a large extent site (and mineralogy) specific (Hinga, 1974; Okalebo *et al.*, 1989).

Muriuki (1979) and Esilaba (1986) have reported a close relationship between Olsen P test levels and crop P uptake in a wide range of soils with diverse characteristics. Crop response to P fertilizer is dependent on other factors such as the soil pH (suitable range of 5.5-7.0 has been suggested by Tisdale and Rucker (1964) for maximum P availability to plants, soil moisture content, P-sorbing capacity of soils and the clay contents. In this context, some Kenyan soils (mainly the luvisols) with low P sorbing capacity respond to phosphate application rates as low as 20 kg P/ha (Okalebo *et al.*, 1989) whereas the nitisols of the highlands, with high clay contents and high P-sorbing capacity, only respond to larger rates of phosphate (Okalebo *et al*., 1991). Information on the soil P test values and the response of maize to fertiliser application may be obtained from Table 31.3.

Table 31.3. An example of soil test data interpretation under major nutrient limited and non-limiting soil conditions based on Kenyan studies of N and P nutrition of maize.

Location	Classification (FAO system)	Soil Test Value				
		pH (in $CaCl_2$)	Clay	C	N	P required[1] ($kg\ ha^{-1}$)
			%			
Sites that responded to N and P application						
Makava Farm, Makueni	Acri-rhodic Ferralsol	4.86	9.2	1.1	0.08	-56
Kyengo Farm, Wamunyu	Haplic Alisol	4.40	---	0.3	0.06	-4
Ithookwe, Kitui	Ferral-chromic Luvisol	5.20	31.1	0.8	0.10	72
Mutua Farm, Katumani	Haplic Lixisol	5.30	---	0.6	0.08	224
Keree Farm, Kakamega	Eutric Nitisol	4.70	53.6	2.8	0.21	981
Kidetok (UGANDA)	Orthic Ferralsol	5.50	17.3	1.6	0.14	31
Kakemega Research Station	Eutric Nitosol	5.10	49.2	3.2	0.29	650
Mean (Standard Deviation)		5.01 (.32)	32.1 (19.4)	1.5 (1.1)	0.14 (.08)	271 (393)
Sites that did not respond to N and P application						
Muguga, NARC	Humic Nitisol	5.72	52.1	3.6	0.41	721
Kitali Top Farm	Orthic Ferralsol	5.19	41.9	2.7	0.24	68
Kabati, Kitui	Acri-rhodic Ferralsol	4.90	14.3	1.0	0.10	-168
Kiboko Research Station	Orthic Luvisol	6.10	15.2	1.2	0.15	-23
Serere Station (UGANDA)	Orthic Ferralsol	5.60	18.8	2.9	0.14	139
Mean (Standard Deviation)		5.50 (.47)	28.5 (17.4)	2.3 (1.1)	0.21 (.12)	147 (340)

[1] ($kg\ ha^{-1}$) from P sorption isotherms

Table 31.4. Critical Al-saturation levels (and ranges) for selected tropical crops (after Anon., 1986).

crop	critical Al-saturation (%)
mung bean	0
soybean	0 - 25
sorghum	15
maize	30
sweet potato	30
Voandzeia subterranea	40
peanut	40
upland rice	40 - 60
cowpea	60
cassava	75

Interpreting of cation data. Continuous cropping in absence of nutrient replacement contributes to the depletion of cations in soils, in part depending on the extent of weathering of soil minerals. Youthful soils, and those rich in 2:1 minerals tend to have adequate levels of K. In respect to the toxic effects of cations in low pH soils, the calculation of % Al-saturation is a useful index to assess the suitability of different crop species for cultivation in specific soils or the liming requirement. The % Al-saturation of a soil may be expressed as:

$$\textbf{Al saturation (\%)} = \textbf{[(Al + H) / ECEC]} \times \textbf{100, or}$$

$$\textbf{Al saturation (\%)} = \textbf{[(Al + H) / (K + Ca + Mg + Na + Al + H]} \times \textbf{100}$$

where the values of the nutrients (and Al + H) are expressed as c.mol/kg soil. When interpreting extractable acidity data, it is important to remember that crop species and cultivars have different tolerances to aluminum in soil. The critical % Al-saturation of different species is presented in Table 31.4. It is important to note that in studies of cations, different chemicals and procedures are used to extract cations in soils (NH_4-acetate, acetic/lactic acids, Olsen procedures). Thus wide variations in the levels of cations in soil are expected from different extraction techniques, resulting in different interpretations. Tekalign *et al.*, devised a guideline for exchangeable cation rating in Table 31.5

Table 31.5. Evaluation of exchangeable cation levels in soils.

Rating	K	Mg	Ca
	---------- mg kg^{-1} ----------		
Very high	> 300	> 180	> 2400
High	175-300	80-180	1600-2400
Medium	50-175	40-80	1000-1600
Low	50-100	20-40	500-1000
Very low	< 50	< 20	< 500

Table 31.5 is based on medium cation exchange capacity soils (± 15 cmol.kg^{-1} soil) common in tropical soils (Cottenie, 1980). From these data it can be surmised that cations may occur in a wide range of relative proportions and that no single extractant dilution will be appropriate in all instances. Users of this manual are advised to be prepared to calibrate their dilution levels to meet specific conditions depending on soil type and operating conditions within the laboratory.

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APPENDIX 1

CONCENTRATION NORMALITY, AMOUNTS OF CONCENTRATED ACIDS AND BASES TO MAKE 1 LITRE OF 1 N SOLUTION

Acid or ` Base	Specific Gravity	Percent by weight	Grams per litre	Approximate normality	ml needed to make 1 litre of 1N solution
Acetic acid	1.05	99.0	1042.0	17.45	58
Ammonium hydroxide	0.90	28.33	255.0 (NH_3)	15.0	67
Hydrochloric acid	1.19	38.0	451.6	12.4	81
Hydrofluoric acid	1.16	50.0	577.5	28.8	35
Nitric acid	1.42	72.0	1024.0	16.2	62
Phosphoric acid	1.69	85.0	1436.0	44.0	23
Perchloric acid	1.66	70.0	1165.0	11.6	86
Sodium hydroxide	1.53	50.0	762.7	19.0	53
Sulphuric acid	1.84	96.0	1742.0	35.5	28

Appendix obtained with permission from: Tekalign T., Haque, I. & Aduayi, E.A. (1991). *Soil, Plant, Water, Fertilizer, Animal Manure and Compost Analysis Manual.* Plant Science Division Working Document 13. ILCA, Addis Ababa, Ethiopia.

APPENDIX 2

CONVERSION FACTORS FOR CONCENTRATION

To convert	Into	Multiply By
μg (microgram)	(g)	1×10^{-6}
me/l	mg %	0.1 × eq. wt
	μg %	100 × eq. wt
	mg/l	eq. wt
ppm	μg/ml	1
	mg/l	1
	μg/g	1
	μg	1
	g/l	0.001
	at wt.	1000
C mol/kg	me/100 g	1
(centimol per kg)	mg/l	1000 × at wt.
	ppm	1000 × at wt.
Molar	ppm	1
Mg/l	mg %	0.1
	μg %	100
	me/l	1/at wt.
	g/l	0.001
	at wt.	0.001

at wt = atomic weight; eq wt = equivalent weight = atomic weight/valence

1 nm (nanometer) = 10^{-9} meter
= 1000 pm (pico meter)
= 1 mu (millimicron)
= 10 Å (10 angstrom)

* Ultra-violet range of spectrum covers 185 - 400 nm
* Visible range of spectrum covers 400 - 760 nm.
* Infra-red range of spectrum covers 760 - 1500 nm.

Appendix obtained with permission from: Tekalign T., Haque, I. & Aduayi, E.A. (1991). *Soil, Plant, Water, Fertilizer, Animal Manure and Compost Analysis Manual.* Plant Science Division Working Document 13. ILCA, Addis Ababa, Ethiopia.

APPENDIX 3

MESH SIZES OF STANDARD WIRE SIEVES

Sieve opening in mm	Standard Mesh Nos.		
	US	British	French
2.00	10	8	34
1.00	18	16	31
0.500	35	30	28
0.420	40	36	-
0.250	60	60	25
0.210	70	72	-
0.149	100	-	-
0.125	120	120	22
0.063	230	240	19
0.053	270	300	-

Appendix obtained with permission from: Tekalign T., Haque, I. & Aduayi, E.A. (1991). *Soil, Plant, Water, Fertilizer, Animal Manure and Compost Analysis Manual.* Plant Science Division Working Document 13. ILCA, Addis Ababa, Ethiopia.

APPENDIX 4.

ATOMIC WEIGHTS

Element	Symbol	Atomic number	Atomic weight	Element	Symbol	Atomic number	Atomic weight
Atinum	Ac	89	227.0278	Gold	Au	79	196.9665
Aluminum	Al	13	26.9815	Hafnium	Hf	72	178.49
Americium	Am	95	243*	Helium	He	2	4.0026
Antimony	Sb	51	121.75	Holmium	Ho	67	164.9304
Argon	Ar	18	39.948	Hydrogen	H	1	1.0079
Arsenic	As	33	74.9216	Indium	In	49	114.82
Astatine	At	85	210	Iodine	I	53	126.9045
Barium	Ba	56	137.33	Iridium	Ir	77	192.22
Berkeliom	Bk	97	247	Iron	Fe	26	55.847
Beryllium	Be	4	9.01218	Krypton	Kr	36	83.80
Bismuth	Bl	83	208.9804	Lanthanum	La	57	138.9055
Boron	B	5	10.8	Lawrencium	Lr	103	260*
Bromine	Br	35	79.04	Lead	Pb	82	207.2
Cadmium	Cd	48	112.41	Lithium	Li	3	6.941
Caesium	Cs	55	132.9054	Lutetium	Lu	71	174.967
Calcium	Ca	20	40.08	Magnesium	Mg	12	24.305
Californium	Cf	98	251*	Maganese	Mn	25	54.9380
Carbon	C	6	12.011	Mendelvium	Md	101	258*
Cerium	Ce	58	140.12	Mercury	Hg	80	200.59
Chlorine	Cl	17	35.453	Molybdenum	Mo	42	95.94
Chromium	Cr	24	51.996	Neodymium	Nd	60	144.24
Cobalt	Co	27	58.9332	Neon	Ne	10	20.179
Copper	Cu	29	63.546	Neptunium	Np	93	237.0482
Curium	Cm	96	247*	Nickel	Ni	28	58.69
Dysprosium	Dy	66	162.50	Niobium	Nb	41	92.9064
Einsteinium	Es	99	252*	Nitrogen	N	7	14.0067
Erbium	Er	68	167.26	Nobelium	No	102	259*
Europium	Eu	63	151.96	Osmium	Os	76	190.2
Fermium	Fm	100	257*	Oxygen	O	8	15.9994
Fluorine	F	9	18.9984	Palladium	Pd	46	106.42
Francium	Fr	87	233*	Phosphorus	P	15	30.9738

APPENDIX 4. ATOMIC WEIGHTS (CONTINUED)

Element	Symbol	Atomic number	Atomic weight	Element	Symbol	Atomic number	Atomic weight
Gadolinium	Gd	64	157.25	Platinum	Pt	78	195.08
Gallium	Ga	31	69.72	Plutonium	Pu	94	244*
Germanium	Ge	32	72.59	Polonium	Po	84	209*
Potassium	K	19	39.0983	Tantalum	Ta	73	180.9479
Praseodymium	Pr	59	140.9077	Technetium	Tc	43	98*
Promethium	Pm	61	145*	Tellurium	Te	52	127.60
Protactinum	Pa	91	231.0359	Terbium	Tb	65	158.9254
Radium	Ra	88	226.0254	Thallium	Tl	81	204.383
Radon	Rn	86	222*	Thorium	Th	90	232.0381
Rhenium	Re	75	186.207	Thulium	Tm	69	168.9342
Rhodium	Rh	45	102.9055	Tin	Sn	50	118.69
Rubidium	Rb	37	85.4678	Titanium	Ti	22	47.88
Ruthenium	Ru	44	101.07	Tungsten	W	74	183.85
Samarium	Sm	62	150.36	Uranium	U	92	238.0289
Scandium	Sc	21	44.9559	Vanadium	V	23	50.9415
Selenium	Se	34	78.96	Xenon	Xe	54	131.29
Silicon	Si	14	28.08555	Ytterbium	Yb	70	173.04
Silver	Ag	47	107.868	Yttrium	Y	39	88.9059
Sodium	Na	11	22.9898	Zinc	Zn	30	65.38
Strontium	Sr	38	87.62	Zirconium	Zr	40	91.22
Sulphur	S	16	32.06				

Values marked * are for the most common isotope.

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APPENDIX 5

SOLUTION CONCENTRATIONS

System name	Abbreviation	Definition
Molar	M	$\frac{\text{gram-molecular weight (mole of solute}}{\text{one litre of solution}}$
Molal	m	$\frac{\text{gram-molecular weight (mole of solute}}{\text{one kilogram of solvent}}$
Formal	F	$\frac{\text{gram-formula weight of solute}}{\text{one litre of solution}}$
Normal	N	$\frac{\text{gram-equivalent weight of solute}}{\text{one litre of solution}}$
Weight per volume percent	w/v%	$\frac{\text{number of grams of solute}}{\text{volume of solvent (mls)}} \times 100$
Volume percent	Vol% or v/v%	$\frac{\text{volume of solute}}{\text{volume of solution}} \times 100$
Weight percent	wt% or w/w%	$\frac{\text{weight of solute}}{\text{weight of solution}} \times 100$
Parts per million	ppm	$\frac{\text{milligrams of solute}}{\text{one litre of solution}}$ or $\frac{\text{milligrams}}{\text{kilogram}}$
Parts per billion	ppb	$\frac{\text{micrograms of solute}}{\text{one litre of solution}}$ or $\frac{\text{micrograms}}{\text{kilogram}}$

from: Tekalign T., Haque, I. & Aduayi, E.A. (1991). Soil, plant, water, fertilizer, animal manure and compost analysis manual. *Plant Science Division Working Document* **13**. ILCA, Addis Ababa, Ethiopia.

APPENDIX 6

STANDARDISATION OF 0.05 M EDTA

Principle. Ethylenediaminetetraacetic acid (EDTA) is not among the class of primary standards compounds. It has tendency to change the chemical composition on standing. Therefore for it be used it has to be standardised by titration against primary standard such as calcium carbonate $CaCO_3$. At pH 10 with eriochlome black T as an indicator.

Reagents

1. 0.05 M EDTA: dissolve 18.6 g of $Na_2EDTA.2H_2O$ in 1ltre of distilled water.
2. Buffer solution pH 10: Weigh out and dissolve 34 g NH_4 Cl and 2 g of Mg-EDTA in 350 ml ammonia solution (sp gr=0.91 g/cm^3) and dilute it with water to 1 litre.
3. Indicator solution: Weigh out 0.5 g of Eriochrome Black T and 4.5 g hydroxylamine HCl and dissolve in a 100-ml ethanol 96% (v/v).
4. 1 M hydrochloric acid: Dilute 83 ml HCl (sp.gr.,1.19 g/cm^3) to 1litre with distilled water.

Procedure. Weigh precisely 0.15 g $CaCO_3$ into a 250 ml erlenmeyer flask. Dissolve in 3.0 ml of 1 M HCl (4, above).**dilute all into 100 ml** and heat to boil for few minutes. Add 10 ml of buffer solution (2, above) to the still hot solution. Add a few drops (4-5) of the indicator solution (3, above). Titrate the solution against the EDTA. It is important to replicate the determination and use the mean titre volume.

Calculation. The concentration of EDTA is calculated as follows

$$M \text{ (mol litre}^{-1}) = w \times 1000 / (v \times 100.1)$$

Where w = weight of the $CaCO_3$ and v = volume of the EDTA

Remarks. Store the standardised solution in plastic container. If excess HCl has been used accidentally, the solution can be neutralised first with 1 M ammonia solution.

APPENDIX 7

STANDARDISATION OF POTASSIUM PERMANGANATE WITH EDTA

Principle. Potassium permanganate tends to decompose upon being exposed to light. It is therefore necessary to standardise the salt before using it to prepare standard solutions

Reagents

1. *0.05 M EDTA.* Dissolve 18.5 g of Na_2EDTA in a 1000 ml volumetric flask and fill to the 1000 ml mark with distilled water.
2. *Buffer solution pH 10.* Weigh out and dissolve 34.0 g NH_4 Cl and 2.0 g of Mg-EDTA in 350 ml ammonia solution (sp gr 0.91-g/cm^3) and dilute it with water to 1 litre.
3. *Indicator solution.* Weigh out 0.5 g of Eriochrome Black T and 4.5 g hydroxylamine-HCl and dissolve in a 100 ml ethanol 96% (v/v).
4. *Hydroxylamine-HCl, 1%.* Dissolve 1.0 g of hydroxylanine-HCl in 100 ml of distilled water.
5. *Sodium sulphite solution.* Dissolve 5.0 g of sodium sulphite in 100-ml distilled water.
6. *4 M ammonia solution.* Dilute 75 ml of ammonia (NH_4OH) and 250 ml distilled water.
7. *Tartrate solution, pH 10.* Dissolve 3.75 g of tartaric acid in 35 ml 4 M ammonia solution and dilute with distilled water to 250 ml.
8. *0.7 M sulphuric acid*. Dilute 40-ml of concentrated sulphuric acid (sp.gr. 1.84 g cm^{-3}) in distilled water to 1 litre

Procedure

1. Weigh 0.24 g of potassium permanganate $KMnO_4$ into a 250 ml Erlenmeyer flask, add 50 ml distilled water and dissolve the salt.
2. Add 7.5 ml of 0.7 M sulphuric acid (8, above) and 11 ml of sodium sulphite (5, above).
3. Swirl until the contents are clear or colourless. Note, if the solution is not yet colourless, add a few drops of the sodium sulphite (5, above)
4. Add 10 ml of 1% hydroxylamine-HCl, 30 ml of the tartrate solution add a few drops (4-5) of the Eriochrome Black T indicator (3, above).
5. Titrate with 0.05 M EDTA, colour change will be from red to blue

Calculation. The relative molecular mass m_x of the potassium permanganate is as follows.

$$\mathbf{m_x = w/(a \times b)}$$

where w = weight of the potassium permanganate (mg), a = concentration of the EDTA (M) and b= volume of the titre (ml)

Remark. If the potassium permanganate is found to have partially decomposed, then, weigh out the m_x/m times the prescribed amount

APPENDIX 8

STANDARDISATION OF ZINC SULPHATE ($ZnSO_4 \cdot xH_2O$)

Principle. Zinc sulphate tends to lose water of crystallisation on storage, it is therefore necessary to standardise the salt before it is used for standard solution. A solution of zinc sulphate is titrated against standardised 0.05 M EDTA at pH 10, using Eriochrome Black T indicator

Reagents

1. *0.05 M, EDTA.* Standardised
2. *Buffer solution pH 10*. Weigh out and dissolve 34.0 g NH_4Cl and 2.0 g of Mg-EDTA in 350 ml ammonia solution (sp gr. 0.91g/cm^3) and dilute it with water to 1 litre.
3. *Indicator solution.* Weigh out 0.5 g of Eriochrome Black T and 4.5 g hydroxylamine-HCl and dissolve in a 100 ml of ethanol 96% (v/v).

Procedure. Weigh out precisely about 0.43 g zinc sulphate $ZnSO_4 \cdot xH_2O$ in clean 250 ml Elrenmeyer flask. Add 100-ml distilled water and dissolve the salt. Add 10 ml buffer solution (2, above) and a few drops of (4-5) of indicator solution (3, above). Titrate with 0.05 M EDTA from a burette. The colour change will be from wine-red to a blue end-point

Calculation. The relative molecular mass (mx) of the used zinc sulphate salt is calculated as follows:

$$m_x = w / (a \times b)$$

where a = concentration of EDTA (M), b = volume of EDTA used (ml), w = weight of zinc sulphate.

Remark. If it is found that the salt is partially effloresced, then adjust the prescribed amount by multiplying with m_x/m

APPENDIX 9

STANDARDISATION OF COPPER SULPHATE ($CuSO_4 \cdot xH_2O$)

Principle. Copper sulphate $CuSO_4.5H_2O$ loses the crystal water on storage, therefore it is necessary to determine the copper concentration before using the salt for standard solution.

Reagents

1. *0.05 M EDTA.* Dissolve 18.6 g of Na_2EDTA in a 1000 ml volumetric flask and fill to litre with distilled water (Standardised).
2. *4 M ammonia.* Dilute 5.0 ml NH_4OH with water to 250 ml.
3. *Murexide indicator*. Dissolve 50 mg murexide in 5.0 ml of water. Filter the suspension (saturated solution)

Procedure. Weigh 0.30 g copper sulphate $CuSO_4.xH_2O$ into a clean 250 ml Erlenmeyer flask. Dissolve it in about 100 ml of distilled water. Add drop-wise about 2 ml of the 4 M ammonia (2), a precipitate is formed, then continue to add the ammonia until the precipitate just redissolves. The colour of the solution turns dark blue. Add a few (4-5) drops of murexide indicator. Titrate the content against 0.05 M EDTA from a burette and record the volume. Colour changes from green to deep blue. It is important to replicate the determination then get the mean titre value.

Calculation. The relative molecular mass m_x of the used copper sulphate salt is calculated as follows

$$m_x = w / (a \times b)$$

where a = the concentration of EDTA (M), b = the volume of EDTA used and w = the weight of the copper sulphate $CuSO_4.xH_2O$.

Remarks

1. Adjust the prescribed amount of the copper sulphate by multiplying with m_x/m
2. Avoid excess concentration of ammonia with respect to the copper concentration as this would result to the formation of copper-tetraamine which will partially decompose the copper-murexide complexes which makes it difficult to observe the colour changes.
3. At the beginning of the titration colour changes from dirty-blue due to the formation of copper-ammonia and copper-murexide complexes. The colour changes to green due to the formation of copper-murexide and copper-EDTA complexes. At the end-point colour finally changes to deep blue caused by mixed colours of free murexide and copper-EDTA

APPENDIX 10

STANDARDISATION OF FERROUS AMMONIUM SULPHATE

Principle. Since the ferrous sulphate loses the crystal water on standing, then, it is necessary that the iron content should be determined before the salt is used for standard solution

Reagents

1. *0.05 M EDTA.* Dissolve 18.5 g of $(NH_4)_2Fe(SO_4)_2 \cdot xH_2O$ in a 1000 ml volumetric flask and fill to the mark with distilled water.
2. *2 M Hydrochloric acid.* Dilute 166 ml HCl (sp. gr.=1.19g/cm^3) with distilled water to 1 litre.
3. *M nitric acid*. Dilute 6.9 ml ofHNO_3 (sp. gr.=1.40-g/cm^3) with distilled water to 1litre.
4. *Sodium acetate buffer solution.* Dissolve 64.6 g of sodium ammonium acetate $CH_3COONa.3H_2O$ in 100 ml of 2 M HCl and dilute with distilled water to 250 ml.
5. *Sulfosalicyclic acid indicator:* Dissolve 2 g sulfosalicyclic acid in 100 ml ethanol 96% (v/v)
6. *Hydrogen peroxide, 30%.* Analytical grade

Procedure. Weigh 0.60 g $(NH_4)_2Fe(SO_4)_2 \cdot xH_2O$ into a 500 ml beaker and dissolve it in 100 ml of 0.1M nitric acid (3, above). Heat to boil and add 5-7 drops of hydrogen peroxide to oxidise Fe^{2+} to Fe^{3+}. Boil **for several minutes** to expel the excess hydrogen peroxide, cool. Add 10.0 ml sodium acetate (4, above) to raise the pH to 2.5. Add 2.0 ml of sulfosalicyclic acid indicator (5, above). Titrate the contents against the 0.05 M EDTA (1, above) and record the volume used. The colour changes from red to yellow. It is important to replicate the determination and calculate the mean value.

Calculation. The relative molecular mass m_x of the ferrous ammonium sulphate salt $(NH_4)_2Fe(SO_4)_2.xH_2O$ used is calculated as follows

$$M_x = w / (a * b)$$

Where a = concentration of EDTA (M), b= volume of EDTA used (ml), w= weight of the salt ferrous ammonium sulphate

Remarks

1. When preparing standard solutions using this partially effloresced ammonium iron (II), adjust the prescribed amount by multiplying it with m_x/m.
2. The boiling of the ammonium ferrous sulphate in acid medium helps to complete the dissolution of Fe^{3+}.
3. At pH 2.5, the yellow colour is intensified due to the hydrolysis of the Fe^{3+}.

Laboratory Methods of Soil and Plant Analysis: A Working Manual

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