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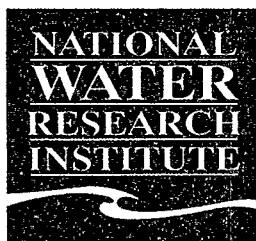
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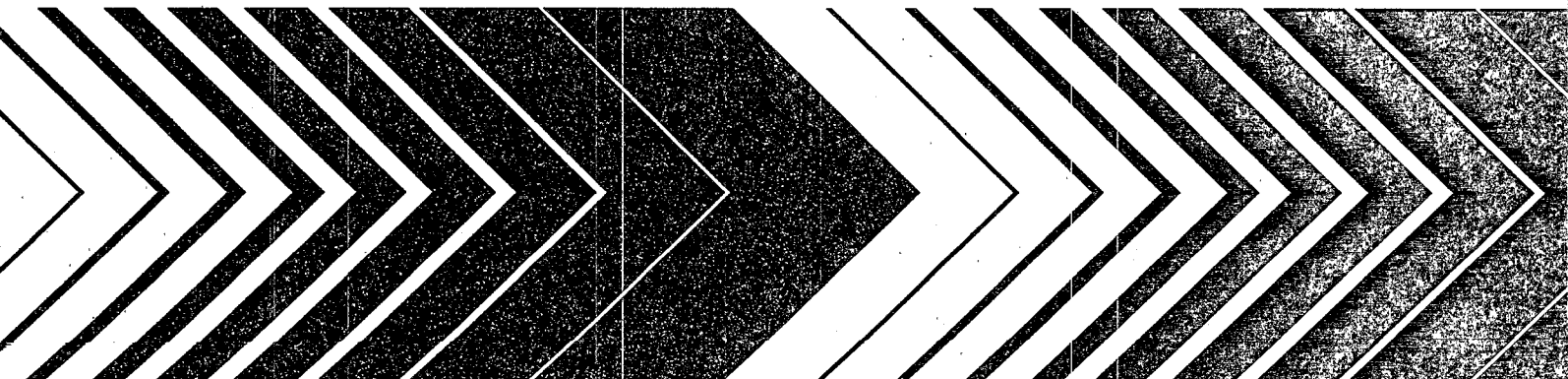
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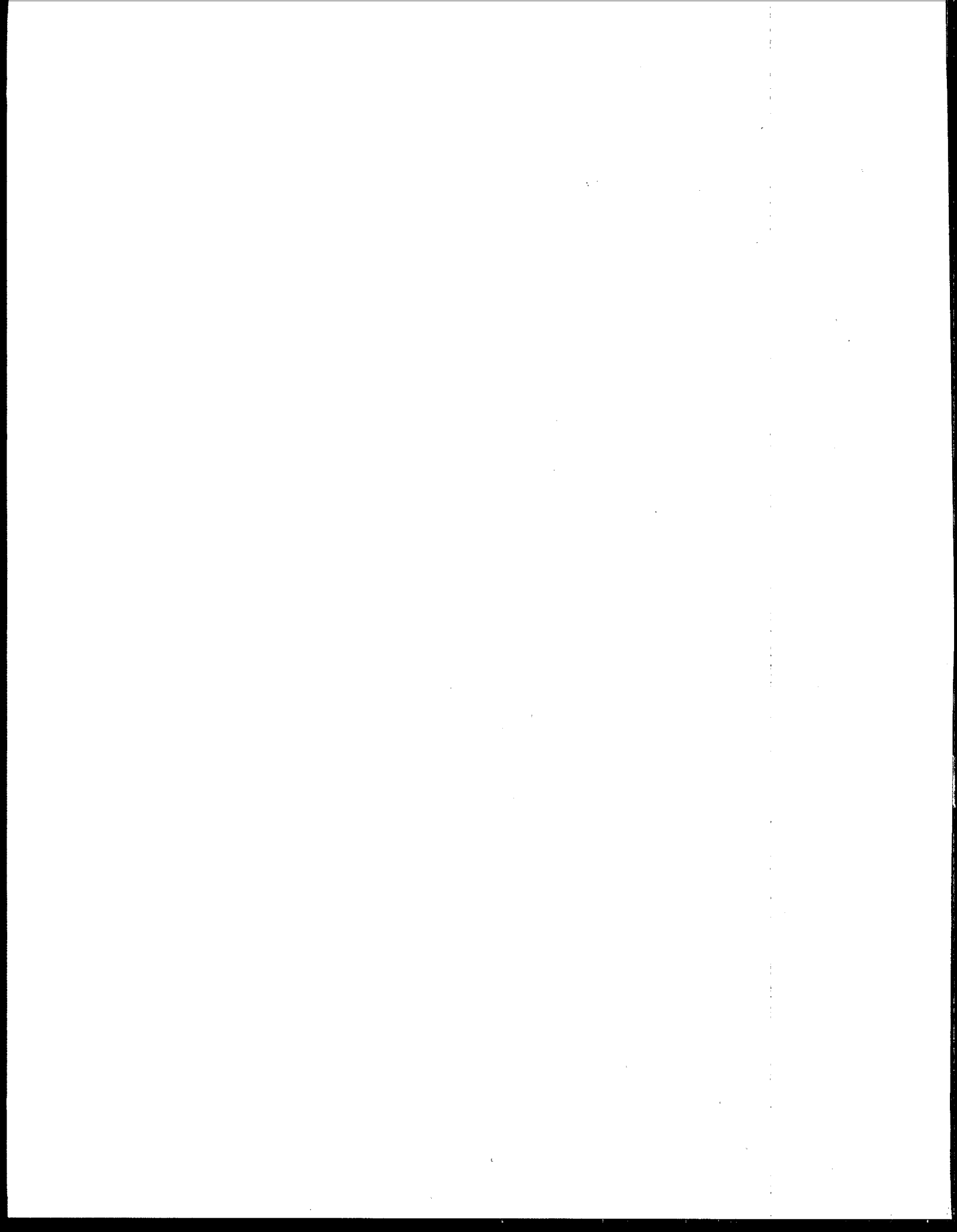


Canada



Laboratory Methods for Soil and Foliar Analysis in Long-Term Environmental Monitoring Programs





Laboratory Methods for Soil and Foliar Analysis in Long-Term Environmental Monitoring Programs

by

**The Ad Hoc Terrestrial Monitoring Work Group of the Quality Assurance Subgroup
Focusing on Monitoring the Terrestrial Effects of Acidic Deposition**

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NOTICE

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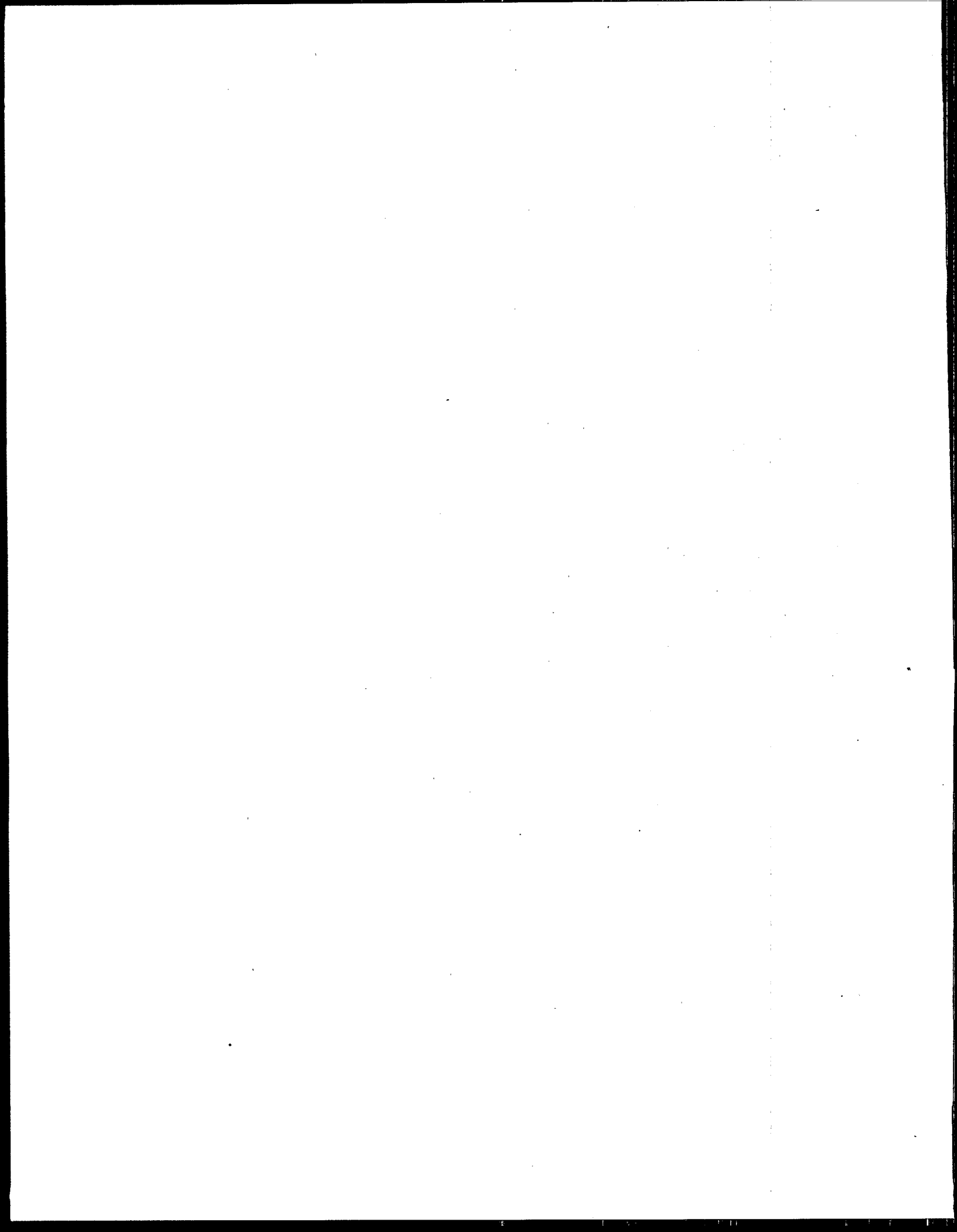
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Analytical Methods for Terrestrial Samples

Introduction

The success of long-term environmental monitoring studies, such as those designed to measure the effects of the long range transport of atmospheric pollutants, will depend on the type of data collected and the comparability of the data over the course of the monitoring period. Data comparability will depend on collection and analysis procedures as well as the natural spatial and temporal variability of the soil and vegetation. The objective of this document is to present methods for the collection, preparation, and analysis of soil and plant tissue samples taken as part of a long-term study to evaluate the effects of acid rain on terrestrial systems.

The objective of the terrestrial monitoring program is to measure real changes caused by acidic precipitation. Unfortunately, these changes can be hidden by natural spatial and temporal variability or variability resulting from errors or changes in the measurement process. Spatial and temporal variability can be addressed through proper sampling designs. Measurement errors can be minimized through quality assurance protocols, such as the development of consistent analytical techniques. It is hoped that this report will assist in selecting and documenting appropriate analytical methodologies.

Quality Assurance and Quality Control

Quality assurance may be defined as "a system of activities whose purpose is to provide to the producer or user of a product or service the assurance that it meets defined standards of quality with a stated level of confidence" (Taylor, 1987). The goal of the quality assurance/quality control (QA/QC) procedures presented in this manual is to ensure that the data collected for a given parameter is of the highest integrity and that the data quality can be evaluated and documented.

To ensure the integrity and comparability of analyses among various laboratories, several quality control procedures have been presented in each of the methods. These procedures deal with the assessment of precision and accuracy and the use of method blanks, quality control preparation samples (QCPS), and quality control check samples (QCCS). Within each method, acceptance limits and frequency of use within an analytical batch of these QC samples has been presented. Further, a suggested run format has been presented showing a potential distribution of the QC samples within the analytical run.

To assess analytical precision, at least one sample should be analyzed in duplicate with each run of thirty samples or less. In sample batches that are expected to have large analyte concentration fluctuations, additional duplicate samples are recommended (recommended rate is 1 duplicate sample per 10 routine samples) to assess precision. To avoid bias associated with position within the run, non-adjacent duplicate analysis is recommended. For example, if the suggested run format indicates to run the duplicate sample after the 16th routine sample (i.e., in the middle of the run), therefore, the associated replicated sample should be selected from the first (sample numbers 1 to 8) or last (sample number 26 to 30) group of routine samples.

Accuracy can be assessed through the use of standard reference materials (SRMs). For many of the methods presented in this manual, SRMs are not available from commercial sources. SRMs are available for total elemental analyses, such as total carbon, in vegetation and soil samples. Accuracy checks, however, for extractable soil parameters (preparation + analysis) can be created by using the median values of large interlaboratory round-robin studies with soil samples prepared

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and shipped strictly for that purpose. Where available, accuracy standards are recommended to be run in the middle of the analytical run.

Method blanks are analyzed to assess if contamination of the sample or sample extracts has occurred. Contamination can occur from numerous sources, such as glassware, filters and reagents used during sample preparation or analysis. It is recommended that 3 or more method blanks be analyzed at the beginning, middle, and end of the run. If contamination is identified within the run (i.e., the method blank has a concentration greater than the instrument detection limit), two courses of action can be taken. First, rerun the blank to ensure that the value is above the instrument detection limit. If the method blank still indicates contamination is present, then all samples between the last "non-contaminated" blank and the contaminated blank should be rerun until no contamination is indicated in the new method blank (i.e., the method blank prepared during the rerun of the routine samples). The second course of action occurs for those methods where significant preparation is involved in the blanks and thus detectable analyte concentrations will occur in the method blanks. In this situation, if all the routine sample concentrations are significantly higher (e.g., 10 times greater) than the blank, analyses should continue with a blank correction occurring on the final sample results using the mean blank value. In cases where the blank and the routine samples are close in measured concentrations, then the decision should be made by the laboratory manager on whether to reanalyze or accept the results.

The QCPS is a matrix matched in-house quality control sample used to monitor accuracy and long-term between-run precision. This sample should be a soil sample that has been collected and prepared in bulk and analyzed numerous times through time and across projects/programs. After each analysis, the resultant analyte concentrations should be incorporated into the database and a new accuracy window developed for that sample. Over a long period of time, the median value or long-term mean may approximate the "true" value for the sample. Ideally, more than one QCPS should be obtained to better characterize the routine analytical range and sample type analyzed (i.e., sandy, silty, clayey, and organic soils). Further statistical significance and value of the QCPS, can be obtained by submitting the sample through interlaboratory round-robin testing. One QCPS is recommended to be analyzed per run.

The QCCS is a matrix matched standard solution containing the analyte of interest at a known concentration in the mid-calibration range. The QCCS is analyzed to verify the calibration curve (i.e., to monitor and correct for instrumental drift) and should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. This sample should be prepared from a different stock solution than that used to prepare the initial instrument calibration standards and should not be taken through any preparation step.

References

Taylor, J.K. 1987. Quality assurance of chemical measurements. Lewis Pub., Chelsea, MI. 328 pp.

Good Laboratory Practices

Introduction

Providing the client with the best possible data of known and acceptable quality (i.e., with a specified probability of being correct) must be of foremost concern in the analytical laboratory. Good laboratory practices may be defined as a group of operations or procedures which comprise quality assurance within the laboratory, and which are integral to achieving and maintaining the high quality of output from any laboratory. These operations and procedures should become the code of behaviour in the laboratory. They apply to workload planning and receiving; data quality objective setting and sample representativeness; documentation; personnel training; sample storage, preservation, and preparation; laboratory cleanliness; equipment maintenance; laboratory safety; instrument calibration; and analytical quality control protocols. As part of the commitment to quality, staff must be aware of their responsibilities, policies and procedures must be documented and distributed, and the channels of communication between the client and laboratory staff must remain open. This chapter is meant as a general guide for laboratory managers and supervisors. It is designed for the laboratory performing routine tests on soil and foliar samples. It does not provide for unusual samples or complex analytical techniques requiring specialized facilities or equipment (e.g., work requiring "clean room" procedures, radioactive or highly toxic substances, etc.).

Workload Planning and Receiving

Workload planning and sample scheduling is necessary to ensure that the client receives his/her results in a timely manner and that perishable samples are analyzed without undue delay (e.g., field moist samples). Any laboratory providing an analytical service must have available space, staff, necessary facilities, and documented procedures for sample reception, log-in, and preservation. The latter may include soil and foliar tissue drying, freezing, refrigerating, freeze drying, and storage. A method of tracking samples as they pass through the laboratory is also required. This is usually done by assigning a unique laboratory number or label to the sample. The protocol should be documented to guarantee consistency between laboratory personnel and within sample types. The number or label must be traceable to the original field sample number, client, and date of receipt at the laboratory. The chain of custody for each sample must be known so that the sample's disposition at any time can be determined. Having the analyst initial a form when samples are removed from and returned to their place of storage and initial the bench sheet after completion of an analysis helps to track the progression of samples through the laboratory.

Data Quality Objectives and Sample Representativeness

The data quality required (sensitivity and coefficient of variation at all levels of detection) must be established by the client at the outset of the study. This allows the project and laboratory

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managers to work together to achieve these objectives. For the data user, it is critical that the sample is representative of the environment under observation. It is the responsibility of the sampler and project manager to provide samples which best represent the environment being measured. For some purposes, a single sample or a homogenized or composite sample prepared from a series of separate samples may be adequate. More frequently, however, the collection of replicate samples from the same sampling location is necessary to obtain a measure of variability in the field. These field replicates must not be confused with laboratory replicates or "sample splits" which are analyzed routinely in the laboratory to provide a measure of analytical precision.

It is the responsibility of the laboratory analyst to ensure that the method provides data of acceptable quality and that this quality is not compromised by the use of inappropriate sampling containers, preservatives, storage facilities, or handling procedures. Moreover, it is important that both the laboratory analyst and client know the limitations of the analytical procedures used.

Environmental studies usually require considerable planning. Both the laboratory manager and project manager or client must have input and assume responsibility for ensuring that the project data quality objectives are met.

Documentation

The importance of properly documenting procedures in the laboratory cannot be overstated. Written sample handling, preparation and analytical procedures, maintenance and safety guidelines, and analytical quality control records should be available. This documentation is required for the laboratory to establish its credibility; however, it does not, in itself, guarantee that the procedures are properly followed or that the data produced are of the quality specified. Training and ongoing evaluation of method performance is also needed.

Staff Training

Laboratory training programs are necessary for all new laboratory personnel and existing staff whose duties in the lab are changing. The theory and rationale for existing procedures should be covered in detail.

Sample Preparation

Soil and foliar sample preservation and storage may consist of refrigeration, freezing, freeze drying, air drying, or oven drying. When the analysis requires field moist samples and the samples cannot be analyzed immediately, refrigeration at 2 to 4° C or freezing at -20° C is recommended. Refrigerated samples should be analyzed within one or two days of receipt. The analysis of field-moist soil samples is often dictated by the project objectives or the tests requested. Irreversible

changes in some parameters can occur as a result of drying, particularly in organic soil horizons (Peverill et al., 1975; Maynard et al., 1987; Kalra and Maynard, 1991). Most methods described in this manual call for dried soil and foliar samples and, therefore, only practices related to their preparation are discussed.

Soils

Samples should be dried on non-metallic trays or plastic sheets in an area free from airborne dust and chemical fumes. Sample drying and grinding/sieving should not be performed in the same room. Large lumps or clods should be broken by hand to facilitate drying. Samples should be dried at 20 to 25° C until a constant mass is attained. Unless specifically requested to do otherwise, samples are disaggregated and sieved. Only the material passing a 2 mm sieve is used for analysis. The > 2 mm material is retained and its weight recorded. The whole sample should be dried and sieved and the < 2 mm portion homogenized and stored in a glass, plastic, or cardboard container with a non-metallic lid.

Some tests, those measuring total amounts of an element or those requiring a very small sample aliquot, require a homogeneous finely-ground sample. Grinding to < 35 mesh (500 µm) is recommended for some of the methods outlined in this manual. When grinding a subsample to pass a specified mesh size, the following steps should be performed:

- The sample should be well mixed before a subsample is removed.
- The subsample should be sieved first to remove all material that is naturally less than the mesh size used.
- The subsample should be ground using an agate mortar and pestle (to prevent Al contamination) for short time periods. Frequent re-sieving is necessary until the whole subsample passes through the sieve. This prevents biasing the sample by discarding any portion of the sample which is difficult to grind, and it prevents over-grinding of minerals. Over-grinding is not a problem if total amounts of an element are measured, but can be a problem for the measurement of extractable Fe and Al (Neary and Barnes, 1993).

Foliar Tissue

Foliar samples should be dried in a forced draft oven at between 70 and 80° C. Lower drying temperatures should be used if volatilization is of concern. Dried samples are ground to pass a 20 mesh (850 µm) sieve. Large samples may be first ground through a Wiley Mill using a 10 mesh (2 mm) sieve and then reduced by quartering and put into an Intermediate Wiley Mill or Tecator Cyclotec (Kalra and Maynard, 1991). These samples may be used for the determination of N, P, K, Ca, Mg, Na, and S. A non-metallic grinder is recommended if Fe, Mn, Cu and Zn are to be measured. Samples should be transferred to non-metallic storage containers and tightly sealed for storage.

Laboratory Cleanliness

Laboratory cleanliness encompasses everything from maintaining an organized workplace within the laboratory to the quality of the air, water supply, and reagents. A well-designed laboratory in terms of workstation location helps avoid accidents and reduces the chance of sample contamination from labware and reagents used for other tests. Shared equipment (balances, centrifuges, water baths, block digesters, stirrers, vortex mixers, shakers, etc.) should be thoroughly cleaned after each use to prevent sample contamination and salt build-up from sample extracting and digesting solutions. Similarly, the sample path in analytical instruments should be thoroughly rinsed/flushed with deionized water after each use. This will extend the life of the instrument and prevent contamination of the next batch of samples analyzed. Burner heads from atomic absorption spectrophotometers should be cleaned after each use to prevent salt build-up. When equipment must be shared, it is advisable that all batches of one test be completed before another batch is started.

When cleaning glassware and plasticware consideration must be given to the expected analyte concentrations in the sample. All new glassware and plasticware should be cleaned with soap and water. A contaminant-free detergent, such as Decon or Acationox, which rinses well is recommended. Three short rinses with distilled/deionized water after washing are preferable to one long rinse. For trace element analysis, labware should be soaked in nitric acid before use. Separate labware should be used for the analysis of soil and foliar tissue. More rigorous cleaning procedures and separation of labware is required if sub-mg/L levels are to be measured.

The quality of the air in the laboratory is important for the safety of the staff and to prevent contamination of the samples. All instruments and equipment should be vented according to the manufacturer's specifications. Instrument vents and fume hoods should be checked on a routine basis so that airborne contamination in the form of fumes or dust is prevented. Soil drying, sieving and grinding should not be performed in the same room used for sample extraction/digestion and analysis. Even the weighing of dried samples can generate a significant amount of dust and should be done in an area removed from sample analysis. Soil samples and vegetation samples should be weighed and analyzed in different locations.

A source of purified water is essential to the analytical laboratory. Laboratories having an on-line supply of purified water should check the conductivity of the water regularly. Periodic checking of the analyte of interest can be done by analyzing the purified water as a blank sample in the run. This might be in addition to the matrix-matched method blanks which are part of each batch of samples analyzed. If water purification (distillation, deionization, etc.) is done by the laboratory staff, regular maintenance schedules and procedures must be documented and followed. A conductivity of below $1 \mu\text{S cm}^{-1}$ for freshly purified water is suitable for most soil and vegetation analyses. If the conductivity of the water is above $2 \mu\text{S cm}^{-1}$, the water should not be used until the system is inspected and cleaned. The conductivity of purified water sitting in carboys for long periods will gradually increase to between 1 and $3 \mu\text{S cm}^{-1}$ as ions are leached from the walls of the

container. If an on-line deionized water tap is present, it may be advisable to discard the first three litres of water at the beginning of each work day (Robarge and Fernandez, 1986). In cases where NH_4^+ or NO_3^- -free water is required, amine-based deionizing cartridges may be a source of error. Cleaning of cartridges with formaldehyde has also been found to contribute to contamination problems.

Reagents must be free of contamination by the analyte. New batches of reagents should be checked prior to their use. Matrix-matched blank solutions prepared with the new reagent should be checked against blanks prepared with the old batch of reagent. Reagents not meeting the purity requirements should be labelled as such and either discarded or stored separately. Special purity requirements for reagents or dilution water must be documented in the standard operating procedure.

Reagent Traceability

All reagents must be dated upon receipt at the laboratory and on opening to prevent prolonged storage of reagents beyond their estimated shelf-life (Robarge and Fernandez, 1986). Prepared reagents should be labelled with the solution name, concentration, date of preparation, and name of person preparing the reagent. In Canada, WHMIS (Workplace Hazardous Material Information System) labelling is a requirement for all bought or prepared reagents. WHMIS legislation also sets strict regulations for the storage of chemicals. The laboratory manager must make sure that all staff receive WHMIS training.

All powders used for primary standard and reagent preparation should be numbered upon receipt and all stock solutions and reagents prepared using that powder should be numbered and have a paper trail back to the batch of chemical used. Similarly, all intermediate and working solutions should be traceable to their source. If certified stock solutions are used instead of neat chemical powders for standard preparation, all solutions prepared from these stocks should be traceable back to the original bottle used. When the stock powder or purchased stock solution is discarded or exhausted, records should be kept and the replacement given a new and unique number upon receipt. This rigorous record keeping will greatly simplify trouble-shooting for problems, such as reagent contamination, sensitivity irregularities, calibration errors, etc.

Equipment Maintenance

An equipment maintenance schedule can extend the life of an instrument, reduce repair costs, and maximize instrument performance. Equipment is often shared by many users in the laboratory. It is advisable, therefore, to assign one person the responsibility of maintaining a given piece of equipment. Although all operators should be trained to run daily safety and performance checks before use and to clean the instrument after use, sharing the routine maintenance of the instrument among operators does not work well. This is especially true for sophisticated instrumentation such

as atomic absorption spectrophotometers, ICP emission spectrometers, ion chromatographs, continuous flow systems, carbon analyzers, and X-ray fluorescence spectrometers. Detailed equipment maintenance procedures, schedules, and performance checks are usually provided by the manufacturer. Below are some general guidelines for maintaining and using equipment most commonly found in the soil/vegetation laboratory.

As much as possible, instruments should be located in areas free from dust and fumes. Although most analytical instruments are sealed and protected from dust and laboratory chemicals, a dust cover is advised when the equipment is not in use, especially in particularly corrosive or dusty environments. Reagents should never be stored on the top of instruments. The level of waste containers located under instruments should be closely monitored. The pressure on pump lines of continuous flow systems should be released when not in use. All filters and traps on atomic absorption spectrophotometers, ICP's, carbon and sulphur analyzers, etc. should be checked, cleaned, and replaced regularly. Ion chromatograph pumps, and sample chambers and lines on all instruments should be rinsed well after use, especially if strong salt solutions are used. The performance of automated shut-down mechanisms should be checked regularly.

Weighing samples and reagents is a daily activity in the soil/vegetation laboratory. Balances are often used intermittently throughout the day by many lab personnel. The balance should always be cleaned after each use. Even when sample or chemical spillage does not occur, the weighing of finely ground soil and vegetation samples will leave a film of dust on the balance. The balance pan should be removed and the whole balance cleaned after each use with a camel-hair or other soft-haired brush. Wiping with a moist cloth should be done only after brushing to prevent scratching and smearing of the balance with moistened sediment.

Balances should be located away from drafts caused by nearby doors, heating and cooling vents, exhaust fans for instruments, fume hoods, centrifuges etc. A balance table is recommended even though the newer four-place balances are particularly stable. If the balance must be located on the laboratory bench, centrifuges, vacuum pumps, stirrers, or any equipment likely to cause vibration, should not be used on the bench at the same time. Balances should be routinely checked for accuracy using a certified set of weights. Occasional recalibration may be necessary. Balances should be turned on in the morning and left on throughout the day.

Centrifuge heads and shakers should be cleaned on a regular basis to avoid salt build-up from extracting solutions. This build-up may not only contaminate other samples but will eventually cause pitting of the metal centrifuge head, weakening it, and making it unsafe. Samples must always be placed in the centrifuge in a manner to ensure that it is balanced and the centrifuge should never be opened when in use.

Aluminum block digesters are often used for strong acid digestions of soil and vegetation. These must be used in a fume hood and all non-aluminum hardware (e.g., screws, handles, rivets, etc.) on either the block itself or side covers should be regularly checked for corrosion by acid fumes. Metal contamination from such corroded surfaces is possible. Controllers for the block should be located outside the fume hood.

Maintenance of pH meters consists primarily of proper electrode care. Combination electrodes should be kept adequately filled with saturated KCl solution. Loose KCl crystals should be present at the bottom of the electrode and air bubbles should not be present. The rubber sleeve should cover the filling hole when the electrode is not in use. Storage buffers and cleaning solutions recommended by the manufacturer should be used. The electrode should be rinsed between samples using a stream of deionized water. Wiping of the electrode with laboratory tissue is not recommended.

Laboratory Safety

A laboratory safety manual should be available and read by all laboratory personnel. In Canada, all laboratory personnel are required to undergo WHMIS training. This training provides information on storage, labelling and use of chemicals, chemical waste disposal, and transportation. WHMIS Material Safety Data Sheets (MSDS) must be available for all chemicals used in the laboratory.

Safe laboratory practices for all the analytical procedures outlined in this manual cannot be covered here; however, some general principles of laboratory safety should be mentioned. Specific safety precautions during an analysis should be outlined in the standard operating procedure. Instrument operating manuals, provided by the manufacturer, usually outline safety requirements and should always be kept with the instrument. Emergency shut-down procedures for instruments should be posted. Safe laboratory practices must never be compromised for the sake of decreasing sample throughput time. Personnel should develop a positive attitude toward safety.

Fire extinguishers, eye wash stations, safety showers, first-aid kits and chemical spill kits should be present and easily accessible in all laboratory areas. Laboratory evacuation procedures should be known and practiced by all staff. Fire escape routes must be marked and kept clear of carts, equipment, gas cylinders, coat racks, etc. Extreme care must be exercised when handling flammable or potentially explosive materials (e.g., acetylides, perchlorates, azides, ozonides, and peroxides). Flammable materials should be stored separately in specially designed metal storage cabinets and carried in safety cans. Concentrated acids and bases should be stored in specially designed vented cabinets and transported in safety bottle carriers. All cylinders must be properly secured and the tank pressure not allowed to drop below the recommended level. Acids and organic solutions must be stored separately. Storing powdered reagents in alphabetical order, a practice commonly seen in the laboratory, should not be done. Oxidizing and reducing agents must not be put together. Staff should receive training in the cleaning of chemical spills and emergency contact numbers should be readily available. First aid and cardiopulmonary resuscitation (CPR) training for staff is also recommended.

The importance of proper analytical technique (e.g., pipetting, preparing solutions with concentrated acids or bases) and the use of personal safety equipment such as lab coats, chemical-resistant aprons, gloves, and eye protection must be emphasized by the laboratory

supervisor. Wearing contact lenses in the laboratory is not advised and open-toed shoes should not be worn. Food and drink should not be consumed or stored in the laboratory. Smoking is prohibited by law.

Equipment or procedures emitting chemical fumes should be vented to the atmosphere. This includes atomic absorption spectrophotometers, ICP emission spectrometers, muffle and combustion furnaces, and microwave ovens. Fume hoods should be used only to perform operations for which they were designed. For example, if perchloric acid is used, a specially designed stainless steel hood without any exposed organic material or sealer and with a proper washdown facility is required. Fume hoods should be washed down regularly and not modified without the manufacturer's approval (e.g., removal of baffles). The ventilation efficiency of the hood should be measured with the sash in its normal position under routine working conditions.

Most laboratory accidents are a result of failing to observe basic safety precautions. Each worker should ultimately be responsible for his/her own safety and each laboratory supervisor should try to maintain a safely designed laboratory and ensure that laboratory staff have access to all necessary safety equipment and information.

Analytical Quality Control

As part of quality assurance within the laboratory, good laboratory practices must include quality control protocols for ensuring that an analytical method is operating as expected and meets the predefined data quality objectives. Continual review of these protocols and performance data will help to identify problems early in the analytical process. Corrective actions can then be taken with minimal loss of data to the client.

Method Development

Method development is the set of experimental procedures designed to measure a known amount of a substance in various matrices. Method development ensures and demonstrates that the extraction of the substance and response of the measurement system to that substance follows a specific behaviour in a predictable, reliable, and stable fashion. The sample matrix used in the development process must represent the type of samples which will be analyzed routinely. The ruggedness and application of a method to the sample matrix for which it is intended must be determined and the predefined data quality objectives met before the method is adopted. All known interferences and shortcomings should be listed in the standard operating procedure and made available to the data user.

A measure of single-operator precision is required before comparison with data obtained by other analysts and other methods. Inter-laboratory comparisons are useful and should be continued even after the method is brought "on-line". When changes or improvements to a method are made, all changes and dates of changes must be documented. An overlap period when the old and new

methods are run concurrently is necessary until the data collected are representative of all types of samples received. Differences in sample values and daily performance checks should be monitored and the methods evaluated. The client/data user must be told how the methods compare so that historical data may still be useable.

Method Documentation

The written procedure should include all sample preparation and clean-up steps, detailed description of the measurement system including instrumental conditions and adjustments, calibration procedures, performance checks, safety practices, performance characteristics, and data limitations (Ontario Ministry of the Environment, 1986).

Calibration

Calibration of the measurement system with more than two standards of known concentration should be performed before the analysis of every batch of samples. It is advisable to prepare calibration solutions from a primary standard. A primary standard is a substance of high purity, with the purity known to within very close limits. These standards should be validated against certified reference standards (e.g., National Institute of Standards and Technology or NIST) and traceable to the validation.

Calibration is performed to establish the relationship between instrument response and analyte concentration on that particular day. Calibration establishes the linearity of the analytical system. Sudden changes in the slope and intercept of the calibration curve from day to day should not be expected and, therefore, the slope and intercept should be monitored with each standardization. If these changes occur, insufficient instrument warm-up time, instrument degradation, reagent quality, or error in standard preparation may be the cause.

Working analytical standards are prepared from stock solutions. The concentration of the stock solution should be high enough that pipetting of less than 1 mL to produce a working standard is unnecessary. Working standards should be prepared daily if their concentrations are less than 1 mg/L or if the standards are prepared in a matrix where prolonged storage may cause changes in the results.

Calibration Performance Checks - Accuracy and Precision

Internal Reference Checks

Quality control check standards (QCCS) should be used to check the accuracy of the calibration. These standards should be prepared from a different primary standard than was used in the creation of the calibration standards, or at least a different batch number of the same primary standard. Using a solution in the low and high end of the calibration range is advisable. Sufficient volumes of each quality control solution should be prepared to last through several batches of

calibration standards. The concentrations of these solutions should be read each time the instrument is calibrated and the measurements should be recorded. This record provides data which can be used to calculate the between-run precision of the measurement system separately from sample preparation precision. Limits on this precision may be set and used to identify calibration problems. In addition to the quality control solutions, a long-term blank comprised of the dilution matrix used to prepare the quality control solutions will provide a check on the zero standard or intercept of the calibration curve and the purity of the reagents and distilled/deionized water used.

External Reference Checks

Externally prepared, certified control solutions, such those obtained from the U.S. Environmental Protection Agency, may also be analyzed periodically to provide an accuracy check. Routine participation in an inter-laboratory comparison study gives the analyst an indication of how well his/her laboratory performs with respect to others.

Interference Checks

An interference in the analysis may occur when a substance other than the analyte is present in amounts sufficient to affect the results. Interferences are often identified during the method development stage and steps to prevent these interferences are built into the procedure. During routine operation, checks may be used to monitor the effectiveness of the methodological safeguards used to prevent the interference. Interference checks should be close to the threshold level of the substance found to affect the results. Good laboratory practices require that the analyst carry out all cross checks available, based on the knowledge of the sample source and matrix, to provide for the accurate identification and quantification of the parameter being measured.

Sensitivity and Baseline Checks

Changes in the sensitivity of the instrument should be monitored periodically (e.g., every tenth sample) by analyzing a standard and comparing the peak height to the original calibration. Any change must be considered when calculating the results. Periodic analysis of a blank, matrix matched to the standards and samples, may be used to monitor baseline drift.

Method Performance Checks - Accuracy and Precision

Method Blanks

With each batch of samples, three method blanks (reagents only without sample) should be carried through the entire procedure. These provide a measure of contamination from all possible sources (reagents, labware, filter paper, handling etc.). The contribution of only the reagents and instrumental analysis to the overall contamination can be determined from the zero standard and

long term blank. The mean blank value should be subtracted from the sample result before reporting the final parameter concentration.

Internal Reference Checks

"Total" elemental analyses are not usually performed on soil samples, with the exception of total C, N, P, and S. For most purposes, specific fractions or forms of elements are extracted. These fractions may have been found to relate well to plant growth or soil genesis. As a result, the accuracy of the method cannot be routinely checked by using spike recoveries. As previously mentioned, the accuracy of the measurement system can be controlled. However, the accuracy or specificity of the extraction/digestion for a particular form of the analyte in the soil must be determined in the method development stage. For total measurements (especially on foliage), spiked samples may be used to measure recovery. In this case, the spike should be twice the endogenous level or ten times the detection limit, whichever is greater. The analyst should note that the chemical form of the element in the spike may be different than the naturally occurring form in the soil/foliage. This, in turn, may lead to invalid conclusions about recovery from the sample. When interferences are indicated by the spike recovery, standard additions may be useful for accurately determining the amount of analyte present in the sample.

The reproducibility of the method between batches prepared on different days may be termed the between-run precision and is measured by replicate control samples. Large amounts of soil or foliar tissue should be collected, dried, and ground as required for the test and analyzed with each run of samples. The sample volume should be sufficient for at least a year, if not more, of routine operation. More than one of these between-run control samples should be prepared to represent the low and high part of the analytical range and different sample matrices (e.g., organic and mineral soil or different vegetation species). If run over a long period, the standard deviation of the results of these samples will provide a measure of between-run precision. Control limits may be set at two or three standard deviations from the long-term mean of the results. Samples falling outside these limits may indicate problems in batch preparation. If changes in the analyte occur due to storage of the control sample over long periods, the change may be detected more easily by plotting the results on a control chart. A systematic increase or decrease in the values over time may indicate an unstable sample, or in the case of soil, a sample which may no longer be homogenous, but which has settled and become sorted. Ensure proper mixing/homogenization prior to weighing the sample each day.

The reproducibility of a method within the same batch of samples prepared on a specific day may be called the within-run precision. Some samples within the batch (e.g., every tenth sample) are prepared in duplicate. The run format should be such that the duplicate samples are not analyzed side by side within the run. This will eliminate possible bias resulting from position in the run. Standard deviations of the duplicates can be determined using the following equation:

$$S = \sqrt{\frac{\sum_{i=1}^n (x_1 - x_2)_i^2}{2n}}$$

where: S = standard deviation of the difference between duplicate pairs,
 n = number of duplicate pairs, and
 $(x_1 - x_2)_i$ = difference of the *i*th duplicate.

Acceptable duplicate data should conform to limits which are based on historical performance.

External Reference Checks

Certified reference soil and vegetation samples are available from NIST (formerly the National Bureau of Standards or NBS) and can be run as an accuracy check. Inter-laboratory comparisons provide a good indication of your performance as it compares to other laboratories. Informal comparisons can usually easily be arranged by contacting the laboratory supervisor. The Council on Soil Testing and Plant Analysis based at the University of Georgia provides a soil and plant laboratory registry which may be used to obtain a list of laboratories doing similar work in Canada and the United States. In Canada, the Expert Committee on Soil Survey (ECSS), Agriculture Canada, Land Resource Research Centre, Ottawa Ontario conducts soil round-robins. Similarly, the QA Subgroup of the Research Monitoring Coordination Committee of the federal Long Range Transport of Air Pollutants (LRTAP) program conducts a foliage round robin. This is organized through the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario. LABEX (Laboratories Exchange Program) is coordinated by the International Soil Reference and Information Centre, Wageningen, the Netherlands. A foliage sample exchange has also been organized in the past by IUFRO (International Union of Forestry Research Organizations), and is also based in Wageningen, the Netherlands.

Method Detection Limits

The method detection limit is usually defined as the smallest amount of analyte which may be measured under routine operating conditions. It is the minimum amount that can be reliably discerned as being different from the blank level. There is some degree of imprecision in measurements at all analyte concentrations and the percent contribution of that imprecision is generally greater at low levels. Considering this, the reported detection limit must take into account this imprecision and should not be reported merely as the lowest readable value above the blank on a particular day. The instrument detection limit may be determined from the standard deviation of the blank value calculated from a large number of runs. The method detection limit, however, may be calculated from the mean standard deviation of either a low level standard or preferably replicate samples at close to blank levels. Generally, three times the standard deviation of the blank or low

level standard is quoted as the instrument detection limit. A minimum number of replicates required for the determination of detection limits should be seven.

Blind Audit Samples

To avoid special care being given to quality control samples and samples which form part of an inter-laboratory comparison, it is often valuable to arrange for blind audit samples to be submitted to the laboratory. These samples may be submitted by the client, laboratory manager, or external organization. They should be received through the normal channels and should be unrecognizable from real samples. This allows a better measure of the performance of a method under routine operating conditions.

Summary

Ultimately, the integrity of one's data depends on the quality of the operations within the laboratory. For this reason alone, the importance of good laboratory practices cannot be overstated. The above discussion provided only a cursory review of any of the topics. It is advisable that anyone embarking on terrestrial monitoring work adequately address the area of good laboratory practices before proceeding. The very nature of long-term environmental monitoring may mean changes in laboratories and/or laboratory staff over the course of the monitoring period. Many researchers have found their data sets incomparable or have found uncertainties in historical data. Frequently, these problems result from improper documentation of laboratory procedures, quality assurance and control, and measures of accuracy and precision. Addressing these areas at the outset of a monitoring study will help avoid future disappointment.

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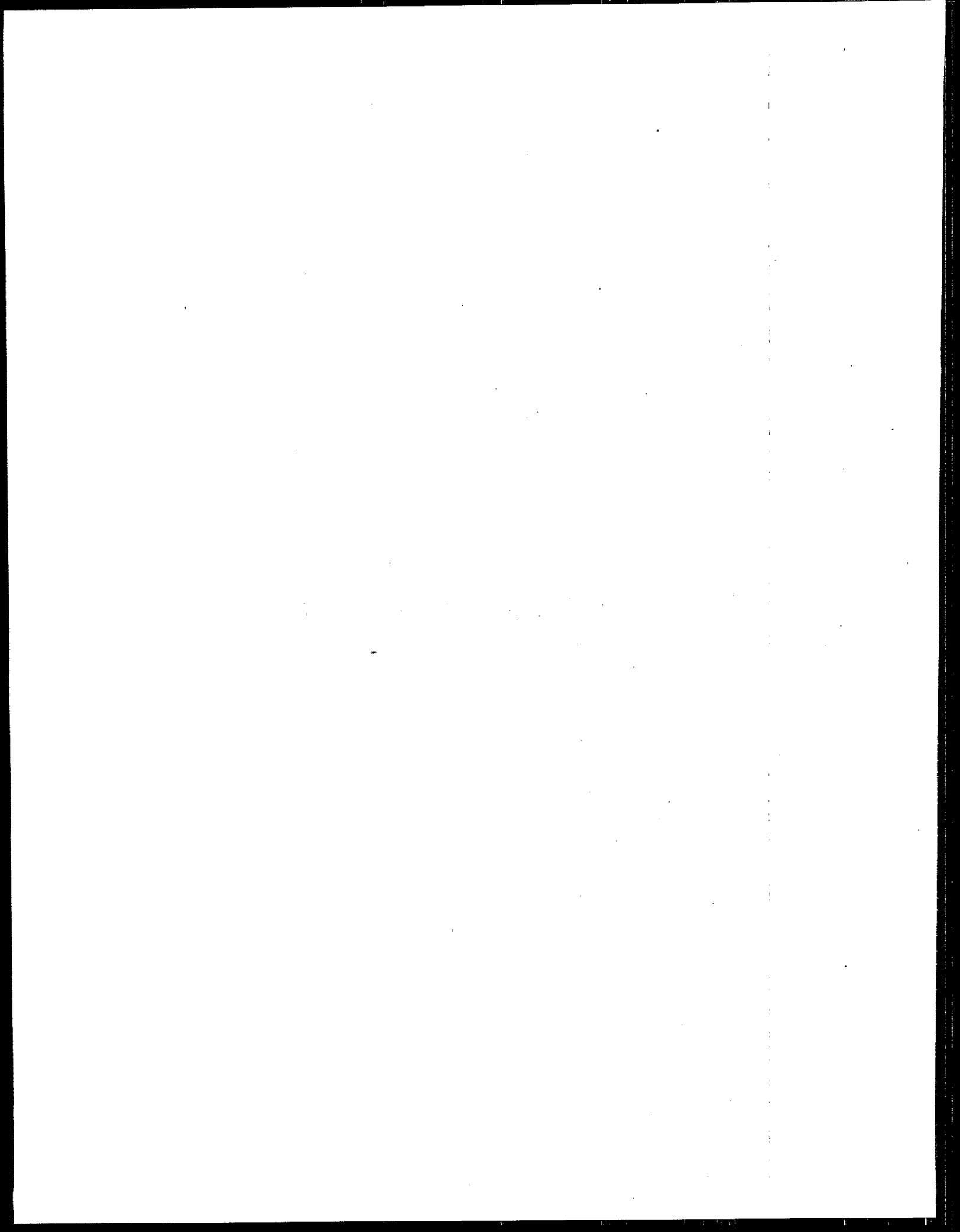
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PLANT TISSUE ANALYSIS



Total Nitrogen in Plant Tissue

Introduction

Nitrogen is one of the five major constituents of living matter. As such, its form and concentration are of major interest when dealing with the growth of terrestrial and aquatic organisms. In nature, nitrogen is found in a number of forms, all of which are interconvertible given the correct chemical and microbiological conditions. Soil N accounts for only a small fraction of N in the lithosphere, and of this fraction, only a very small proportion is directly available to plants since greater than 90% of the N is in the organic form. Plants obtain most of their N from inorganic ions, NO_3^- and NH_4^+ , that comprise less than 1% of the total N.

Atmospheric deposition of NO_x can directly affect the total foliar N concentrations. Further, foliar N content changes may be brought about by indirect effects to the forest ecosystem, such as a result of acid deposition. Therefore, the measurement of total N in plants is suggested for long-term terrestrial monitoring programs, such as LRTAP.

Review of Methods

Total N in plant tissue is usually measured by either a wet oxidation (Kjeldahl method) or dry oxidation (Dumas method) procedure (Bremner and Mulvaney, 1982). Considerable modifications have been proposed for these methods (e.g., Nelson and Sommers, 1980). More recently, near infrared reflectance spectroscopy (NIRS) and LECO combustion have been used as alternatives to the conventional chemical analysis for total N determination in foliage (Tsay et al., 1982; Wessman et al., 1988). These are not in widespread use, however, and they involve expensive equipment. For these reasons, chemical wet oxidation techniques are most frequently used.

The principle of the Kjeldahl and Dumas techniques have been thoroughly discussed in several reviews (Bremner, 1965; Nelson and Sommers, 1980; Bremner and Mulvaney, 1982). The Dumas methods do not quantitatively recover many nitrogenous compounds (e.g., heterocyclic compounds) and the automated N analyzer based on the Dumas method has not been widely used (Bremner and Mulvaney, 1982). The Kjeldahl procedure is more commonly used and modifications of the original Kjeldahl methods have extended the scope of the procedure. The total Kjeldahl N method involves the digestion of the sample with H_2SO_4 to convert organic N to NH_4^+ -N followed by distillation of the digest with strong alkali to liberate NH_3 . Various modifications to the distillation and measurement of the liberated NH_3 have been proposed. In addition, various methods for the direct measurement of NH_4^+ in the Kjeldahl digests have also been used. Highly refractory organic N compounds or compounds containing N-N or N-O linkages are not completely recovered by the Kjeldahl digestion (Bremner and Mulvaney, 1982).

Reference Method

The reference method uses the Kjeldahl acid digestion in the presence of a catalyst which converts organic nitrogen to inorganic ammonium. The measurement of N is done using alkaline distillation to liberate NH_3 which is measured by acid titration. The digestion is carried out either on a hot plate or in an aluminum digestion block.

Summary of Method

Vegetation samples are digested by the regular Kjeldahl technique using a K_2SO_4 - $CuSO_4$ catalyst in an aluminum block digester with internal heaters and temperature control. Determination of NH_4^+-N is done by an automated system (Tecator Kjeltac 1030) determining NH_3 liberated by distillation of the digest with 40% NaOH. The NH_3 is absorbed in unstandardized H_3BO_3 and ammonium borate is formed. The borate is titrated back to H_3BO_3 using a standard strong acid (HCl).

Interferences

After digestion, samples must not be allowed to cool in the digestion block as NH_3 will be lost from the $(NH_4)_2SO_4$ formed by the digestion.

Safety

Protective clothing and safety glasses should be worn when handling strong acids. The digestion blocks should be located in the fume hood and, if possible, the temperature controller should be located outside the fume hood. The equipment should not be left unattended. The preparation of the NaOH should also be done in the fume hood. The NaOH pellets should be added very slowly to the water and in very small portions due to the intense exothermic reaction that occurs.

Apparatus and Equipment

- digestion block, 20 place, Tecator System 20 1050 or equivalent, with programmable temperature controller.
- distillation and titration apparatus, Kjeltac Auto 1030 Analyzer or equivalent.
- glass digestion tubes (295 mm x 40 mm), 250 mL to fit block, appropriate to sample and solution volume used.
- balance, accurate to 0.001 g.

Reagents and Consumable Materials

- sulphuric acid, H_2SO_4 , concentrated, reagent grade (96%).
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- catalyst, Kjeltab tablets or equivalent. Each tablet contains 3.5 g K_2SO_4 and 0.4 g $CuSO_4$.
- hydrochloric acid, HCl, standard acid 0.01 M.
- boric acid, H_3BO_3 , reagent grade powder.
- ammonium chloride, NH_4Cl , reagent grade powder.

- methanol, reagent grade.
- sodium hydroxide, NaOH, reagent grade pellets.
- sodium hydroxide solution, 40%--Dissolve 10 kg of NaOH in 15 L DI water.
- bromocresol green.
- bromocresol green solution--In a 100 mL volumetric flask dissolve 0.100 g bromocresol green in methanol. Dilute to volume with methanol.
- methyl red.
- methyl red solution--In a 100 mL volumetric flask dissolve 0.100 g methyl red in methanol. Dilute to volume with methanol.
- receiving solution (Tecator, 1985)--Dissolve 100 g H_3BO_3 in DI water and dilute to 10 L. Add 100 mL bromocresol green solution. Add 70 mL methyl red solution. Add 5 mL of 40% NaOH solution.
- recovery check solution, 5,000 mg-N/L--In a one litre volumetric flask dissolve 19.0927 g NH_4Cl in DI water. Dilute to volume.

Calibration and Standardization

Before analyzing the digested samples, distilled water blanks are run on the Kjeltec 1030 Analyzer until a constant reading of HCl is obtained. A 5 mL aliquot of a recovery check solution containing 5,000 mg-N/L is analyzed to check the recovery. Recovery should be within $\pm 10\%$.

Procedure

- Step 1 - Weigh 0.250 g of plant material (20 mesh) into a digestion tube.
- Step 2 - Add 10 mL concentrated H_2SO_4 to the tube and mix by swirling.
- Note:** This step should be carried out in a fume hood.
- Step 3 - Heat tubes at 200° C in the digestion block until very black (approx. 30 minutes).
- Step 4 - Add one catalyst tablet (Kjeltab).
- Step 5 - Heat tubes at 200° C for 15-20 minutes until the Kjeltab dissolves.
- Step 6 - Increase the block temperature to 300° C and heat for 30 minutes.
- Step 7 - Raise the temperature to 425° C and heat tubes until the sample turns a turquoise green. Digest samples for 20 minutes.

Step 8 - Remove the digestion tubes from the block and allow to cool for about 5 minutes.

Note: Do not allow to cool in the heating block as NH_3 from the $(\text{NH}_4)_2\text{SO}_4$ formed by digestion will be lost if heated.

Step 9 - Add approximately 30 mL DI water and mix well until sample is in solution.

Step 10 - Dilute to approximately 100 mL with DI water.

Step 11 - Follow instructions for the operation of the Kjeltec Auto 1030 Analyzer (Tecator, 1985).

Step 12 - Set the alkali pump to deliver 25-30 mL of 40% NaOH.

Step 13 - Titrate the sample with 0.01 M HCl.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger. It is recommended that two or more accuracy standards be prepared with each batch of samples. These provide a check on total between-run precision (digestion and distillation/titration).

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

Report total N as percentage on a dry-weight basis to the nearest 0.01% using the following formula:

$$N \% = \frac{(\text{mL sample} - \text{mL blank}) \times \bar{N} \times 1.401}{\text{weight (g) of dry soil}}$$

where: \bar{N} = normality of HCl titrant solution.

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Total Sulphur in Plant Tissue

Introduction

Sulphur is an essential element for all biological systems. Historically, its importance as a plant nutrient and reports of widespread deficiencies, especially in sub-humid areas of intensive leaching has made it a routine measurement in some laboratories. Most of the sulphur in surface soils occurs in combination with organic matter (Freney and Williams, 1983). Sulphate is the most oxidized form of S and most easily taken up by plants and microorganisms (Blair, 1971). Sulphate (SO_4^{2-}) is taken up by plants, reduced to S^{2-} and used to form S-containing amino acids and other reduced-S compounds (Stewart et al., 1983). Most plants contain approximately as much S as phosphorus.

Concern over the long-range transport and deposition of SO_4^{2-} in precipitation has lead to increased monitoring of the S status of soils. The effects of SO_4^{2-} adsorption and desorption on soil cation leaching has also been the subject of much research. Fundamental to studying the effects of strong acid precipitation on terrestrial and aquatic systems is an understanding of the behaviour of S in plant and soil systems. The measurement of total S in plant tissue is, therefore, suggested for terrestrial monitoring programs, such as LRTAP.

Review of Methods

It has only been within the last 15 years that the difficulty of accurately measuring S in soils and vegetation has been overcome. Recent advances in analytical techniques have resulted in the accurate and precise measurement of S in various types of soil and plant materials (Dick and Tabatabai, 1979; Hogan and Maynard, 1984; Nieto and Frankenberger, Jr., 1985; Maynard et al., 1987). Several methods are routinely used for the determination of S in environmental samples. These may be divided into two groups, namely, those involving wet oxidization of the sample and those which involve direct sample analysis (Hogan and Maynard, 1984).

Methods available for the wet oxidation of organic materials are well documented (Beaton et al., 1968; Tabatabai, 1982; Blanchar, 1986). Acid and alkaline oxidation are the most common (Blanchar et al., 1965; Tabatabai and Bremner, 1970), as they are dependable, accurate and relatively rapid (Blanchar, 1986). Full recovery from an acid digestion usually requires the use of perchloric acid. The danger associated with its use and the special facilities required have meant that, until recently, acid digestions have been avoided. The recent adaptation of microwave ovens for use in the laboratory has led to the development of microwave acid oxidation digestion techniques for foliage which successfully use hydrogen peroxide in place of perchloric acid. Many analysts still, however, prefer the safer, more rapid, dry combustion techniques.

The wet oxidation technique converts S to SO_4^{2-} and produces a solution which can be analyzed by a variety of methods. Turbidimetry is insensitive, lacks precision and is subject to numerous interferences (Beaton et al., 1968). Colourimetric methods such as the methylene blue technique (Technicon, 1972) also have limited application in soils and plant analysis because of interferences by major nutrient cations (Maynard et al., 1987). The colourimetric method developed by Johnson and Nishita (1952) was found to be the most sensitive and accurate of the colourimetric procedures.

Recent publications (Hogan and Maynard, 1984; White and Douthit, 1985; Novozamsky et al., 1986) have shown that the accurate and precise measurement of S is possible by inductively coupled plasma-atomic emission spectrometry (ICP-AES) in a range of environmental samples. Precision

is estimated at $\pm 2\%$. Results of analysis of NIST plant material and sediments demonstrated that the precision and accuracy obtained by ICP-AES are equal to or better than any other technique currently used. The ICP-AES has several advantages over other methods. It is rapid, flexible, has a dynamic range, is free from interferences and permits simultaneous multielement analysis. These factors make it a preferred method of analysis for laboratories possessing ICP-AES capabilities.

Busman et al. (1983) successfully determined total S in plant material using a combination of ion chromatography following combustion of the foliage in an oxygen flask.

Direct analysis of the sample may be done by combustion of the sample at elevated temperatures (generally at 1000°C or higher) and measuring the liberated SO_2 by an infrared detector. Examples of instruments that combust the sample include the LECO combustion furnace, Carla-Erba combustion furnace, or the Fisher S analyzer. These methods require little or no sample preparation for the determination of total S.

Additionally, X-ray fluorescence may be used to quantify S in plant tissues along with many other elements. This method has been used for the measurement S in a wide variety of plant materials including lichens (Tomassini et al., 1976). Preparation of vegetation samples for analysis is done by pressing the dried, ground foliage into a pellet using a wax filler. The method is rapid but requires between one and two grams of dried sample. This may limit the analysis of low volume samples. Further, the expense of the instrumentation may make this technique an unfeasible option for many laboratories.

The LECO S analyzer was originally developed for the determination of S in steel, but because of its simplicity, speed, and convenience, it has been adapted for use in soil and plant analysis (Tabatabai, 1982). An initial evaluation of this method by Tabatabai and Bremner (1970) showed total S results to be unsatisfactory for research that required accurate and precise determinations. More recent studies have shown that the LECO analyzer equipped with an infrared detector was capable of providing rapid, accurate analysis of total S in plant material (Hern, 1984; Jackson et al., 1985). Work at the Ontario Ministry of the Environment has found that the infrared detection system on the LECO gave unacceptable results for vegetation and organic soil samples. The LECO analyzer with iodometric titration for the measurement of S was found to give better recovery and precision. A Fisher S analyzer, using a similar theory as the LECO, was also found to provide rapid, accurate analysis of total S in plant material (Guthrie and Lowe, 1984).

Reference Method

The reference method for total S in soil samples is dry combustion in a LECO sulphur analyzer with infrared detection. The analysis of S by LECO-S analyzer has been chosen as a reference method because it is widely used in North America and has been used successfully by some laboratories for both soil and foliage samples.

Summary of Method

An air-dried and finely ground sample of soil or a pre-ashed vegetation sample is heated with an accelerator to 1600°C in a stream of oxygen. The released sulphur is converted to sulphur dioxide and is detected by infrared detector (Hern, 1984).

Interferences and Shortcomings

Large amounts of carbon can prevent proper ignition of the sample. With incomplete combustion a poor recovery of S may be obtained. This may be overcome by reducing the sample size and adding LECO Iron Chip Accelerator plus tin. Preashing foliage and highly organic soils at 475 to 500° C for two hours is usually done, but preliminary studies should be done on the type of samples to be analyzed to determine whether a loss of S occurs during this ashing. Preashed samples also require accelerator to overcome interference from residual carbon.

Moisture deposits on the walls of the delivery tubes or the surface of the dust filter will absorb SO₂. This may be overcome by using magnesium perchlorate between the dust filter and the original drying tube.

Chlorine in concentrations of less than 1% does not interfere. If titration is used to detect S, the halogens, iodine, chlorine and fluorine may darken the solution in the titration vessel by the formation of interhalogen compounds. Poor recoveries may result. A trap inserted in the delivery tube and filled with 20 mesh antimony metal will prevent these interferences.

Interferences from nitrogen may be overcome by increasing the oxygen flow rate to 1.5 litres/minute.

Safety

Normal safety precautions should be taken when using high-frequency combustion furnaces.

Protective clothing and safety glasses should be worn when handling reagents. Heat resistant gloves may be needed when placing samples in the furnace. The furnace must be adequately vented and protected from human contact and combustible materials. Gas cylinders should be bolted or chained in an upright position.

Fumes of magnesium oxide are toxic. Magnesium perchlorate is a fire and explosion hazard if it comes in contact with organic materials.

Apparatus and Equipment

- sulphur analyzer with infrared detector, LECO model SC-132, or equivalent.
- balance, accurate to 0.001 g.
- muffle oven, capable of maintaining 500 ± 5° C.
- desiccator and desiccant, P₂O₅.
- LECO scoop.
- LECO crucibles and covers.

Reagents and Consumable Materials

- oxygen, high purity.
- compressed air, if needed.
- anhydrous magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$, 10-20 mesh, or equivalent desiccant specified by manufacturer for drying gases after combustion and prior to detection.
- magnesium oxide, MgO , reagent grade powder, low in sulphur.
- accelerators, vanadium pentoxide, iron chips, and/or copper metal

Calibration and Standardization

Set up the instrument according to the LECO operating manual. In general, the instrument should be calibrated at least once a day or once per batch of samples, whichever is more frequent. Use either NIST (formerly NBS) reference materials or standards supplied by the manufacturer and approved by the laboratory or QA manager. The concentration range of the standards must be representative of the C concentrations expected in the soil samples. A minimum of a two-point calibration curve should be used. Use of a NIST standard reference material as an initial calibration check is highly recommended.

Some suggested calibration standards and reference samples include: LECO brand iron powder (0.036% S), LECO coal calibration standard (2.56% S), NIST (NBS) coal reference material (1.89% S), SU-1A nickel-copper-cobalt ore (9.35% S), and CCU-1 reference material (35.4% S).

Ensure that the anhydrous is dry and the dust filter is clean. Prior to analyzing samples, the instrument is conditioned by running low level calibration standards until the results are stabilized to within 5%. Once stable, three blank crucibles (accelerator only) are analyzed followed by three standards.

Procedure

- Step 1 - Weigh out approximately 3 g of sample into a ceramic crucible. Record sample weight.
- Step 2 - Ash sample at 500°C for 2 hours in the muffle oven. Cool sample in desiccator. Re-weigh cooled sample and record weight of ashed sample.
- Step 3 - Preset power settings on induction furnace according to the manual.
- Step 4 - Ignite sample in the crucible for the suggested time period (either given in the operating manual or as determined through method development work for the particular sample type being analyzed).
- Step 5 - Take sulphur measurement reading.

Quality Control

Precision

One sample from each batch should be analyzed in duplicate. Within-run precision is determined from duplicates based on relative percent difference (RPD) between the samples with an acceptance limit of a $RPD \leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Two blank crucibles (accelerator only) are analyzed before the run and one blank crucible is also analyzed in the middle and at the end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Suggested Run Format

MB, MB, Samples 1 to 8, QCPS,
Samples 9 to 16, MB, DUP,
Samples 16 to 25, SRM,
Samples 26 to 30, MB.

where: MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

Infra-red system: % S is read directly from the instrument. The sample weight, as different from the standard, is taken into account on some instruments.

Results are reported to two significant figures. Results are read to the nearest 0.001%. Results should be blank corrected.

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P, Mn, Fe, Al, Ca, Mg, and K in Plant Tissue

Introduction

Phosphorus

Phosphorus is a naturally occurring element essential to plant growth and microorganism activity. In acid soils, phosphorus is found primarily in combination with iron and aluminum oxides and oxyhydroxides whereas in alkaline soils, calcium phosphates predominate. Plant available phosphorus occurs almost exclusively as orthophosphate in the soil solution.

Phosphorus levels in the environment are increased significantly by man's activities. Acid deposition may impact on soil and affect the availability of phosphorus to plants. Therefore, measuring phosphorus levels in plants may provide information on phosphorus availability. Increased soil acidity may produce increased aluminum availability. In turn, aluminum toxicity is often manifested in the plant as a phosphorus deficiency.

Manganese

Manganese is essential in plant nutrition and controls the behavior of several other micronutrients. Apparently, the most important function of manganese is related to the plant's oxidation-reduction processes. Manganese appears to participate in the oxygen-evolving system of photosynthesis and also plays a basic role in the photosynthetic electron transport system. Chloroplasts are the most sensitive of all cell components to manganese deficiencies and react by showing structural impairment.

Tissue testing is important in determining the plant manganese content since soil analyses alone are not very reliable in diagnosing the manganese supply to plants. Excesses of manganese can have a toxic effect on plants which is interrelated with other elements, particularly iron.

The manganese content of plants depends on plant characteristics and the pool of available manganese in the soil. Generally, the most readily available forms of manganese are found in a waterlogged acid soil. Manganese toxicity in some field crops might be expected on acid soils with pH values of 5.5 or less and high manganese levels. Acid deposition can, therefore, increase manganese availability.

Iron

Iron is considered the key metal in energy transformations needed for syntheses and other life processes of cells. Iron deficiencies affect several physiological processes and, therefore, can retard plant growth. On soils rich in soluble iron, excessive iron uptake by plants can produce toxic effects. Plant injury due to iron toxicity is most likely to occur in strongly acid soils, acid sulphate soils, and flooded soils.

Acid soils tend to be higher in soluble inorganic iron forms than either neutral or calcareous soils. The concentration of iron in soil solutions within common soil pH levels generally ranges from 30 to 550 $\mu\text{g/L}$, whereas, in very acid soils, it can exceed 2000 $\mu\text{g/L}$. In acid anaerobic soils, Fe may be toxic to plants whereas in alkaline, well-aerated soils, low concentrations of soluble iron species may lead to iron deficiencies in the plant. Almost all cases of iron toxicity and deficiency in plants are considered to be the result of the soil factors governing iron solubility. These properties, in turn, are affected by acid deposition which can alter soil pH and, thus, iron availability to the plant.

Aluminum

Aluminum is a common constituent of all plants although the content varies greatly depending on soil and plant characteristics. There is some evidence that low levels of aluminum can have a beneficial effect on plant growth by controlling colloidal properties in the cell. Aluminum injury or toxicity is often reported for plants grown in acid soils. In soils with pH values below 5.5, aluminum mobility increases sharply. The mobile aluminum very actively competes with other cations for exchange sites resulting in impaired nutrient uptake and transport in the plant.

Aluminum toxicity is also frequently associated with increased levels of iron, manganese, and other heavy metals which are readily available in acid soils. Aluminum excess in plants is known to reduce internal calcium transport and even induce a calcium deficiency in the plant.

Calcium

Calcium is an essential element for all life forms and enhances biological productivity in plants. Calcium imparts rigidity to the cell wall and is necessary to growth. It is generally absorbed by roots by ionic exchange and is thought to affect absorption of other cations and anions. Calcium is essential for maintenance of selective ion transport in cellular membranes and minimizes root injury resulting from sodium and hydrogen ions. A deficiency of calcium results in the disorganization of cellular membrane structure.

In most plants the calcium ion is generally immobile and usually does not move out of older, lower leaves to younger, upper leaves. Therefore, symptoms of deficiency show at extremities of the plant in regions of new growth. Calcium uptake and availability depends on the pH of the soil.

Magnesium

Each chlorophyll molecule contains one atom of magnesium, that is, 2.7% of the weight of the molecule. Magnesium is an activator for many enzymes. The magnesium ion may exist in high concentrations as magnesium sulphate or chloride since these molecules are highly soluble. Magnesium can occur in toxic concentrations if soils are low in calcium but this condition is rare.

In contrast to calcium, magnesium is mobile within the plant. If deficient, magnesium moves from older to younger leaves. Therefore, visual symptoms are exhibited by the older leaves having interveinal yellowing or chlorosis. Magnesium availability decreases in the soil from a pH of 6.5 down to approximately 4.5.

Potassium

Potassium is one of the three most essential nutrients for plants along with nitrogen and phosphorus. The potassium ion is highly mobile within the plant. Potassium, like magnesium, is required for the proper functioning of plant enzymes. The presence of potassium in the plant has also been found to aid in the uptake of other nutrients, namely, anions such as NO_3^- , and in their movement within the plant.

A potassium deficiency can occur in a variety of soils and may be unavailable to plants even when present in the soil. The most characteristic symptom of deficiency is that of tip and marginal scorch of the most recently matured leaves. Potassium deficiency has sometimes been associated with an accumulation of molybdenum in the plant leaves. Potassium availability decreases from pH 6.0 to 4.5.

Review of Methods

The two most common decomposition procedures are wet oxidation and dry ashing. Wet oxidation is the destruction of organic matter by high temperature acid digestion. Common acids used include sulphuric, nitric, hydrochloric, and perchloric acids usually in some combination of two or three. Sulphuric acid is not recommended for use when digesting tissue high in calcium. Relatively insoluble calcium sulphate may form and possibly reduce concentrations of other elements by coprecipitation. Perchloric acid, when hot, is a reactive oxidant and can react with explosive force when brought into contact with highly oxidizable compounds. Therefore, extreme care is required when using this acid. Perchloric acid is often used with nitric acid to speed up the digestion reaction. Further, a combination of perchloric, nitric, and sulphuric acids can be used. Various procedures with this combination of three acids have been developed with the most effective combination being a 1:1:3 perchloric:sulphuric:nitric acid mixture. Exact temperature control is required to avoid the danger of explosion. Temperature control can be maintained through the use of temperature-controlled digestion blocks. The AOAC Official Methods of Analysis (1984) recommends a perchloric-nitric digestion for Ca, Cu, Fe, Mg, Mn, K and Zn determinations in plant tissues. If quantification is to be performed by inductively coupled plasma (ICP) spectroscopy, it is recommended that the perchloric acid be present in minimal quantities.

Wet oxidation can also be carried out under elevated pressures in a Parr bomb (Sung et al., 1984) or in sealed ampules placed into an autoclave at 125° C under pressure (Vigler et al., 1980). Newer methods exist for rapid microwave oven digestions applying the same digestion principles as those applied under elevated pressure conditions.

A combination of hydrochloric-nitric acid can be used but is not usually strong enough to completely digest the plant sample. However, if the sample is first ashed to remove carbon, the hydrochloric-nitric mixture can be employed to dissolve the remaining ash. The ashing temperature is usually kept below 500° C to prevent volatilization losses. In contrast, the temperature must not be too low or incomplete organic matter destruction will occur. In some cases, ashing aids (catalysts or accelerators) may be used to assist in the sample decomposition.

Upon completion of the sample preparation, quantification of the plant nutrients can be performed by ICP or AAS (atomic absorption spectroscopy). If ICP is selected, a vacuum spectrometer is required for sulphur analysis. ICP has high speed, multielement capacity, and computer control capabilities. AAS, while as sensitive as ICP, is more time consuming since analysis is performed one element at a time instead of simultaneously as in the ICP. Further, AAS cannot be used to analyze for sulphur or phosphorus.

Reference Method

A dry ashing technique is the reference method due to the difficulties with wet digestions, such as a loss of calcium with sulphuric acid and the danger of explosion with perchloric acid. This method is appropriate for all the elements being studied (Al, Fe, Ca, Mg, P, Mn, and K). The shape and size of ashing vessel can affect ashing efficiency. A high walled, open vessel is recommended. Silica, pyrex, or well glazed porcelain vessels can be used. Vycor should be used instead of pyrex if boron analysis is required. The muffle furnace temperature must be raised slowly to prevent flaming of the sample. Highly carbonaceous tissue may require an ashing aid such as magnesium nitrate or nitric acid. The ash, after complete organic destruction has occurred, should be white and free of black carbon particles. The ash is combined with a nitric-hydrochloric acid mixture and

heated to dissolve the ash. This will help to ensure release of aluminum and iron. During the acid digestion, hydrogen peroxide (H_2O_2) is added to ensure that manganese oxides are solubilized.

Final elemental quantification should be performed by ICP-AES using a vacuum spectrometer.

Summary of Method

A weighed sample is ashed at 500°C for three hours. The ash is heated in a nitric-hydrochloric acid digestate for four hours. Hydrogen peroxide is added and the volume reduced to 1 mL. The solution is made up to volume and analyzed by ICP.

Interferences and Shortcomings

Volatilization losses can occur on ashing leading to low results. This can be minimized by sequential temperature ashing.

Sequential ICP is less prone to interferences than simultaneous ICP. Spectral overlap can occur but can be compensated for by an interelement correction factors after monitoring and measuring the interfering element. Scattered light, emission from the torch, and several other factors can produce background emission leading to erroneously high results. ICP has the capability of measuring the background intensity at a selected distance adjacent to the analytical line and correcting the reading by background subtraction.

Safety

Normal laboratory safety practices should be observed. Protective clothing and safety glasses should be worn especially when handling HCl , HNO_3 , and H_2O_2 .

Apparatus and Equipment

- crucibles, porcelain.
- watchglasses.
- polystyrene, centrifuge tubes, 15 mL.
- polyethylene dropping bottles.
- muffle furnace, capable of heating to 500°C .
- hot plate, capable of heating to 100°C , or equivalent.
- top-loading balance capable of weighing to 0.01 g.
- balance calibration weights, 3-5 weights covering expected range.
- inductively coupled plasma atomic emission vacuum spectrometer with computer and auto-sampler.

Reagents and Consumable Materials

- nitric acid, concentrated (specific gravity 1.41)--Ultrapure grade, Baker Instra-Analyzed or equivalent.
- hydrochloric acid, concentrated (12 M HCl, specific gravity 1.19)--Ultrapure grade, Baker Instra-Analyzed or equivalent.
- hydrogen peroxide, 50%.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- stock solutions for: potassium, calcium, magnesium, phosphorus, iron, aluminum, and manganese.
- argon, oxygen-free.

Calibration and Standardization

To correctly calibrate and standardize the ICP, refer to operating manual for the instrument. Calibrate by analyzing a calibration blank (0 mg/L standard) and a series of at least three additional standards within the linear range of the instrument. Calibration standards should be prepared in the extraction solution. If an ICP is used, a multi-element standard may be prepared and analyzed. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded.

Procedure

- Step 1 - Weigh 0.50 g of vegetation into a crucible using a top-loading balance.
- Step 2 - In a muffle furnace dry and ash the sample at 150° C for 15 minutes, at 250° C for 60 minutes and at 500° C for 3 hours.
- Step 3 - Wet the sample ash after cooling with DI water.
- Step 4 - Add 1 mL of concentrated HNO₃ and 3 mL of concentrated HCl.
- Step 5 - Place on a hot plate at 35° C (or low setting) for 10 minutes, then raise temperature to just below boiling.
- Step 6 - Cover the crucible with watchglass and digest for four hours. Remove crucible and wash watchglass with DI water adding washings to crucible.
- Step 7 - Add 2 drops H₂O₂ and return to hot plate at 90-95° C.
- Step 8 - Reduce volume to 1 mL by evaporation.
- Step 9 - Transfer to 15 mL centrifuge tube washing crucible with DI water. Dilute to 10 mL.

Step 10 - Mix sample well.

Step 11 - Analyze samples by ICP.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks are taken through the entire digestion procedure to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. Blank concentrations should be negligible and less than the instrument detection limit. Results are blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

The calculations for all the metals are as follows:

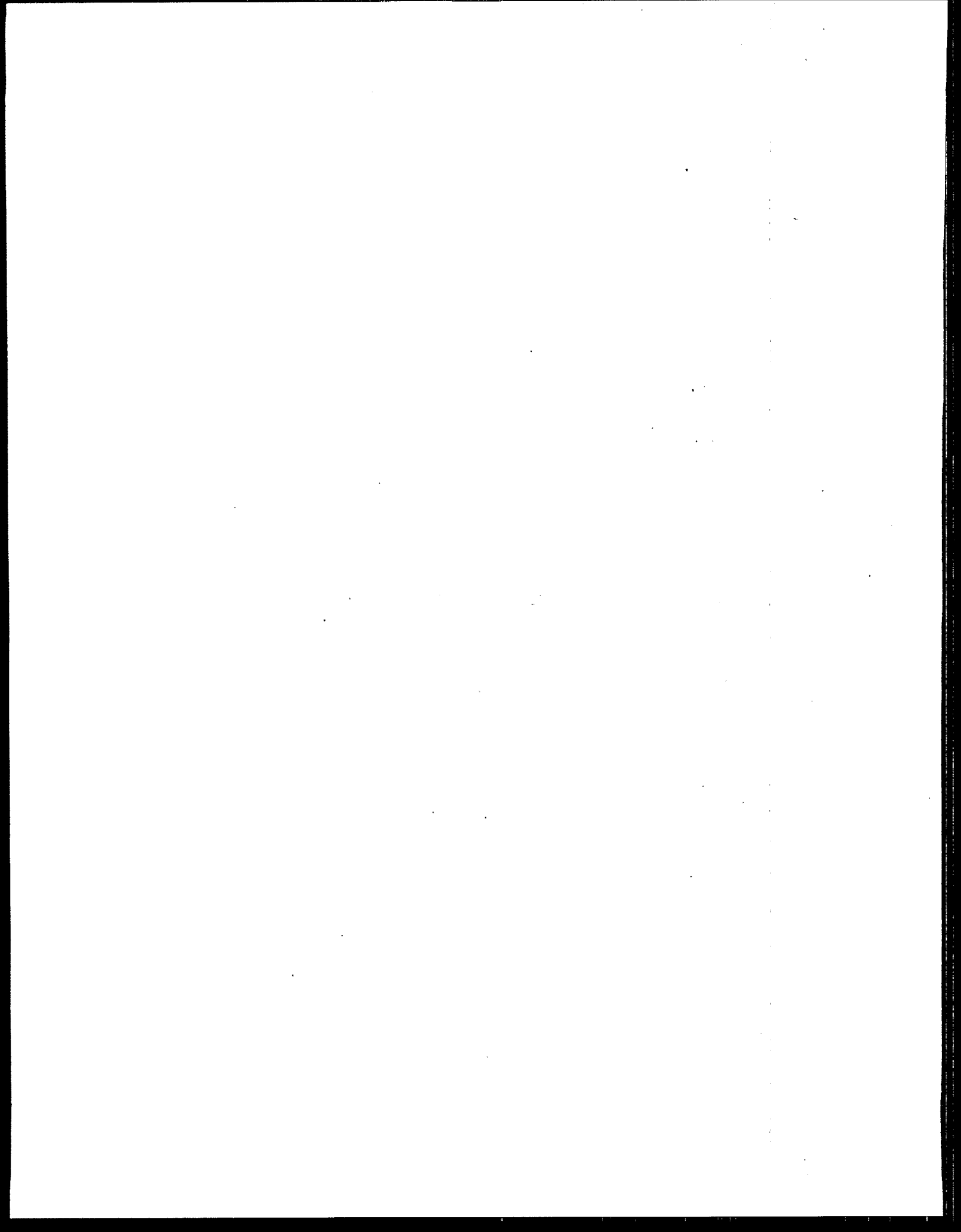
$$\text{Metal } (\mu\text{g/g}) = \frac{\text{metal } (\mu\text{g/mL}) \text{ in solution} \times 10 \text{ mL}}{0.5 \text{ g}}$$

All sample results are method blank subtracted and should account for any dilution factors.

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SOIL ANALYSIS



Particle-Size Analysis

Introduction

Particle-size analysis (PSA) is a measurement of the size distribution of the individual mineral particles in a soil sample (Gee and Bauder, 1986). Particle-size analysis provides fundamental data that can be used for many purposes by many different scientific disciplines. Soil scientists use PSA data in soil classification, evaluation of field texture, quantification of clay movement in soil horizons, determination of the relationship of parent material to the solum, chemical adsorption properties, base exchange capacity, water retention, unsaturated hydraulic conductivity, permeability, aeration, and soil plasticity. Engineers can evaluate tillage properties, potential rates of siltation in waterways, and determine the suitability of soil materials for foundations, landfills, roadways, sewage disposal, etc. using PSA data. Geologists and fluvial geomorphologists use PSA in the evaluation of sedimentation and alluvial properties.

Particle-size analysis characterizes particles with sizes ranging from boulders (>60 cm diameter) to clays (<0.002 mm diameter). A breakdown of the particles by Canadian Soil Survey Committee (CSSC) and the United States Department of Agriculture (USDA) nomenclatures and the defined size limits of their mean diameters are as follows (McKeague, 1978):

<u>CSSC Nomenclature</u>	<u>USDA Nomenclature</u>	<u>Mean diameter size range</u>
clay, fine		0.0002 mm or less
clay, coarse		0.002 to 0.0002 mm
	clay	0.002 mm or less
silt, fine		0.005 to 0.002 mm
silt, medium		0.02 to 0.005 mm
silt, coarse		0.05 to 0.02 mm
	silt	0.05 to 0.002 mm
sand, very fine	sand, very fine	0.1 to 0.05 mm
sand, fine	sand, fine	0.25 to 0.1 mm
sand, medium	sand, medium	0.5 to 0.25 mm
sand, coarse	sand, coarse	1.0 to 0.5 mm
sand, very coarse	sand, very coarse	2.0 to 1.0 mm
	gravel, fine	10.0 to 2.0 mm
	gravel, coarse	76.0 to 10.0 mm
gravel		76.0 to 2.0 mm
cobble	cobble	250.0 to 76.0 mm
stone	stone	600.0 to 250.0 mm
boulder	boulder	600.0 mm or greater

In general, particles with mean diameters greater than 2 mm (gravel or larger) are determined in the field by visual volume estimation and the sand, silt, and clay fractions are determined in the

laboratory. More accurate quantification of the gravel and cobble fractions can be obtained through sieving and weighing of the sample before and after the removal of these individual size fractions. Since soil scientists are predominantly concerned with the sand, silt and clay fractions, the remainder of this discussion focuses on particles less than 2 mm in diameter.

Review of Methods

Particle-size analysis can be divided into three different phases: (1) sample pretreatment, (2) sample dispersion, and (3) weight contribution of each size fraction to the total sample weight. Each phase is comprised of several different processes which are discussed separately.

Sample Pretreatment

Numerous pretreatments have been developed to achieve complete aggregate dispersion in samples. The pretreatments are primarily for the removal of cementing and binding agents such as organic matter, iron oxides, carbonates, and soluble salts. A brief discussion of each pretreatment follows with a more complete discussion presented in Gee and Bauder (1986).

The effect of organic matter on sample dispersion varies greatly with different soil types. Organic matter acts as a binding agent among particles giving the soil the appearance of having a coarser texture than if just the mineral components were analyzed. Organic matter is most commonly removed using hydrogen peroxide (H_2O_2). Hydrogen peroxide is added to the soil until the organic matter is decomposed as generally indicated by a lack of effervescence from the sample. Other oxidants that have been used include sodium hypochlorite ($NaOCl$), sodium hypobromite ($NaOBr$), and potassium permanganate ($KMnO_4$) (Gee and Bauder, 1986).

Iron oxides, such as hematite and goethite, can form strong binding agents among soil particles as either discrete crystals or coatings on particle surfaces (Gee and Bauder, 1986). Iron oxide removal usually involves the reduction and solubilization of iron using Mehra and Jackson's (1960) sodium dithionite-sodium citrate-sodium bicarbonate (DCB) method. This procedure consists of multiple washings with the DCB solution until the soil is gray (gleyed), and subsequent washings with sodium citrate and/or sodium chloride to remove all iron from the system, saturate the exchange sites with sodium, and flocculate the sample. Iron oxides are an intricate part of the mineralogical composition, their removal can change the particle-size distribution and lead to erroneous interpretations of other soil chemical properties that are commonly related to PSA contents (El-Swaify, 1980). The procedure should, therefore, be used with caution.

Carbonates are commonly removed from the soil by washings with dilute 0.2 N HCl, 1 N HCl, or an acidified sodium acetate (1 M NaOAc, pH 5). Sodium acetate is recommended because it is not as harsh as HCl and saturates the exchange sites with sodium. Once again caution should be exercised. Limestone and dolomite particles can be removed resulting in a change in particle-size distribution and textural classification of the soil (Kilmer and Alexander, 1949).

In alkaline soils, soluble salts of calcium, magnesium, and sodium may be present in concentrations high enough to cause particle flocculation. The addition of sodium-based chemical dispersants (to be discussed) further hinders aggregate dispersion by increasing the salt content. Therefore, the salts must be removed prior to sample dispersion. Removal of excess salts can be accomplished by multiple washings with deionized water. Gee and Bauder (1986) suggest that the washings should be continued until the leachate salt concentration drops below 10 mM.

Sample Dispersion

Almost all of the analytical methods for the determination of particle-size distribution require complete sample dispersion prior to quantifying the individual size fractions. Without complete dispersion, PSA results are biased to the coarser fractions since various sized aggregates are measured and not individual soil particles. Sample dispersion can be performed by mechanical (including electrical), chemical methods, or a combination of both.

Common mechanical methods for soil dispersion include: (1) the use of a "milkshake" mixer, (2) shaking overnight on a reciprocating shaker, or (3) ultrasonic dispersion of the sample. The "milkshake" mixer and overnight shaking processes rely on shearing action and turbulent mixing of the sample to disaggregate the soil while the ultrasonic dispersion separates particles through cavitation (Gee and Bauder, 1986). Particle fragmentation is a concern with these methods due to their inherently violent nature. Mechanical dispersion techniques are often combined with chemical dispersing agents to ensure complete sample dispersion.

Chemical dispersion agents work on the principle of particle repulsion. The addition of the dispersing agent elevates the zeta potential by saturating the exchange sites with a monovalent cation, such as Na^+ or NH_4^+ (Gee and Bauder, 1986). Numerous dispersing chemicals have been used, including ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium silicate (Na_4SiO_4), sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$), and several sodium phosphate compounds such as sodium hexametaphosphate, sodium polyphosphate, and sodium metaphosphate. Certain types of chemical dispersion, however, may cause mineral destruction and dissolution.

Quantification Methods

Sand Fractions

Sands are separated from the silts and clays by wet sieving after sample dispersion. The wet sands are oven-dried, passed through a nest of separatory sieves, and weighed to determine the individual sand fraction contents. The success and simplicity of the sieve method has meant the development of very few other methods for the measurement of the sand fraction. Six additional methods to quantify sand fractions mentioned in the literature include the use of the optical microscope, computer-assisted image analysis, visual accumulation tube, sedimentation balance, elutriation, and hydrometer.

Optical microscopy allows for direct observation of the sample with sand fraction percentages determined by grain counting techniques. Additional information about the sand grains, such as colour, shape, and surface morphology, is also obtained and can be preserved via photographs. The major drawbacks of this method are operator fatigue and the extensive time requirements to perform sufficient grain counts to satisfy counting statistics (Yamate and Stockham, 1979).

Another method using the grain counting principle is computer-assisted image analysis (Graf, 1979a). Complete dispersion of the sample is a key factor in this analysis since counting is performed by contrast of the individual grains and against the background. If the sample is not completely dispersed, the computer combines the overlapping grains and count them into a larger size fraction. Since the system is computer operated, the elimination of operator fatigue is a major advantage of the method. This method is still experimental, yet it may have potential for future PSA application.

The remaining four methods for measurement of the sand fraction are based on the settling of grains in a liquid medium. The rate at which different particles settle is directly related to their size (radius). Falling particles follow Stokes' law in which the terminal fall velocity of the particle is defined as follows:

$$v = \frac{g(q_s - q_l) d^2}{(18\eta)}$$

where v = velocity (cm/sec),
 g = gravitational acceleration (cm/sec²),
 q_s = particle density (g/cm³),
 q_l = liquid density (g/cm³),
 d = particle diameter (cm), and
 η = liquid viscosity (g/cm * sec).

Stokes' law assumes that the particles are smooth, spherical, and noninteractive with each other, that terminal velocity is reached immediately at the start of the settling process, and that the viscosity of the liquid controls the rate of settling. Separation of the various particle sizes can be achieved by homogenization of the soil suspension and decanting all that remains above the plane $z = -h$ (in cm) after a given time t (in sec) as follows (Gee and Bauder, 1986):

$$t = \frac{18 \eta h}{[g(q_s - q_l) d^2]}$$

Settling times for silt and clay fractions are provided in Tables 1 and 2.

Table 1. Time required for particles to settle to a depth of 10 cm^{a,b}.

Temperature (°C)	0.02 mm hr:min:sec	0.005 mm hr:min:sec	0.002 mm hr:min
20.0	0:04:48	1:14:13	8:00
20.5	0:04:34	1:13:19	7:54
21.0	0:04:31	1:12:26	7:49
21.5	0:04:28	1:11:34	7:43
22.0	0:04:25	1:10:43	7:38
22.5	0:04:22	1:09:53	7:32
23.0	0:04:19	1:09:04	7:27
23.5	0:04:15	1:08:15	7:22
24.0	0:04:13	1:07:28	7:17
24.5	0:04:10	1:06:41	7:12
25.0	0:04:07	1:05:56	7:07
25.5	0:04:04	1:05:11	7:02
26.0	0:04:01	1:04:27	6:57
26.5	0:03:59	1:03:43	6:52
27.0	0:03:56	1:03:01	6:48
27.5	0:03:53	1:02:19	6:44
28.0	0:03:51	1:01:38	6:39
28.5	0:03:48	1:00:58	6:35
29.0	0:03:46	1:00:18	6:31
29.5	0:03:43	0:59:39	6:26
30.0	0:03:41	0:59:01	6:22

a = assuming $q_s = 2.65 \text{ g/cm}^3$, adjusted for q_i and η for temperature variations.

b = from USDA/SCS, 1992.

Table 2. Sampling depths in cm for the clay fraction (<0.002 mm) for given temperature and settling times^a.

Temperature (°C)	4.5 hours	5.0 hours	5.5 hours	6.0 hours	6.5 hours
20.0	5.82	6.47	7.11	7.76	8.41
20.5	5.89	6.55	7.20	7.86	8.51
21.0	5.96	6.63	7.29	7.95	8.61
21.5	6.04	6.71	7.38	8.05	8.72
22.0	6.11	6.79	7.47	8.14	8.82
22.5	6.18	6.87	7.55	8.24	8.93
23.0	6.25	6.95	7.64	8.34	9.03
23.5	6.33	7.03	7.73	8.44	9.14
24.0	6.40	7.11	7.83	8.54	9.25
24.5	6.48	7.20	7.92	8.64	9.36
25.0	6.55	7.28	8.01	8.74	9.46
25.5	6.63	7.36	8.10	8.84	9.57
26.0	6.70	7.45	8.19	8.94	9.68
26.5	6.78	7.53	8.28	9.04	9.79
27.0	6.85	7.62	8.38	9.14	9.90
27.5	6.93	7.70	8.47	9.24	10.01
28.0	7.01	7.79	8.57	9.34	10.07
28.5	7.09	7.87	8.66	9.45	10.23
29.0	7.16	7.96	8.75	9.55	10.35
29.5	7.24	8.05	8.85	9.65	10.46
30.0	7.32	8.13	8.95	9.76	10.57

a = assuming $q_s = 2.65 \text{ g/cm}^3$, adjusted for q_s and η for temperature variations.

b = from: USDA/SCS, 1992.

The visual accumulation tube is a convenient and simple method to use but is restricted to sand fraction quantification (Schiebe et al., 1981). The researcher simply pours the sand into a long water-filled column and at specified times measures the height of the accumulated sands at the bottom of the tube. The individual fraction heights are converted into percentages by dividing by the total sand column height. The major difficulty with this method is determining the exact height of the column since a flat surface rarely exists in the settled sands.

Sedimentation balances work by measuring the weight of the settled particles from the soil suspension by placement of the weighing pan within the settling column. Siebert (1979) reported excellent correlations between the sedimentation balance and the optical microscope using glass beads, the hydrometer (to be discussed) on geologic samples, and the pipette (to be discussed) on

marine sediments. Complications may exist if the weighing pan is suspended into the suspension from above due to particle settling on balance parts other than the weighing pan.

Elutriation methods, i.e. constant flow systems, depend upon rising currents of water with different velocities to separate particles (Kilmer and Alexander, 1949; Beavers and Jones, 1966). With decreasing current velocity, finer particles are carried to the top of one collection container and transferred to another container (with a different flow velocity) while the coarser particles settle in the initial collection container. Once the particles are separated, particle contents are determined by drying and weighing of the separated fractions. Failures in this system result from the excessive time required (up to 48 hours) to separate clay fractions, clogging in transfer tubes by larger particles, difficulty in maintaining a constant flow velocity causing sedimentation. Beavers and Jones (1966) found that if properly maintained, the elutriator produced clay contents within $\pm 2\%$ of the results obtained by the pipette method.

The hydrometer, although designed primarily to determine silt and clay fractions, can also be used to determine sand contents. The hydrometer and a similar measurement instrument, the plummet, are usually used to determine total sand content and not individual sand fractions. Both instruments work on the principle that density differences occur as a result of particle settling. Density differences change the buoyancy of the suspension resulting in different hydrometer or plummet readings, that are directly related to the various particle sizes, at different times. Problems with this method include difficulty in reading the hydrometer, sample disturbance during instrument insertion, particle settling on the hydrometer surface, and particles settling below the hydrometer leaving an area of decreased density. The increased speed of analysis, however, may override these relatively small errors depending upon operator need and final data usage (Kilmer and Alexander, 1949; Gee and Bauder, 1986). Liu et al. (1966), compared the hydrometer with the pipette method and found good correlations ($r = 0.899$ to 0.980) in clay contents between the two methods in a wide variety of soils from eleven states with the hydrometer method yielding slightly greater clay contents than the pipette method.

Silt and Clay Fractions

Most PSA method development has been devoted to quantifying the silt and clay fractions. New methods were developed to decrease the extensive time required to obtain the results using earlier methods. Some of these earlier methods included: optical microscopy, decantation, centrifugation, elutriation, the use of floats and the manometer, pipetting, and the hydrometer method. The majority of the earlier methods, excluding grain counting by optical microscopy, were based on particle settling following Stokes' Law.

One of the earliest methods used to quantify silt and clay fractions was through repeated decantation, i.e., the Atterburg method (Stein, 1985). This method involves shaking a dispersed soil into suspension, and the removal of the supernatant containing particles of the desired size and finer until a clear supernatant was obtained. Decantation can also be used in the separation of sands from the silt and clay fractions. Although this method is effective for the separation of silt

and clay fractions, its major drawback is the extensive time required for the analyses. Kilmer and Alexander (1949) reported that about 7 days were required to separate eight to ten samples.

A variation on the decantation method was the use of a centrifuge to shorten the settling time. Centrifugation markedly increased the gravitational acceleration (g) as defined in Stokes' Law thereby shortening the time required for particle settling. After centrifuging, the supernatant was decanted and the soil resuspended into solution. The process was repeated until a clear supernatant was obtained.

The manometer and float techniques measure particle-size distribution based on changes in suspension density. The manometer measures resultant pressure head changes within the suspension due to the settling of particles below the measurement arm. Various floats of different densities were placed in the suspension and the time was recorded for a given density float to reach a certain depth in the suspension. Alternatively, the depth to which the float sank after a specified settling time was recorded. The time or depth measurement was converted to particle-size percentages using variations of Stokes' Law. The manometer and floats both had the inherent problem of remaining in suspension throughout the measurement period which allowed for particle sedimentation on instrument surfaces, thereby affecting the final results. Furthermore, the manometer was difficult to read due to small pressure changes and floats of good quality were difficult to obtain (Rose, 1954).

The most common technique for PSA of silt and clay fractions is the pipette method. This method is perhaps the "standard" method to which most other PSA techniques have been compared. The pipette method consists of bringing the dispersed sample into suspension, allowing an appropriate settling time, and sampling to a specified depth using a pipette of known volume. The extracted aliquot is dried, weighed, corrected for the weight contribution of the dispersion agent, and converted into weight percent silt or clay. A variation on the pipette method, the Andreason pipette, employs the same technique except that the pipette is permanently mounted in the sedimentation cylinder (Siebert, 1979). Problems arise from the use of the Andreason pipette due to settling of the sample on the pipette and a disruption of the particle settling pathway.

With advances in electronic and X-ray technologies and improvements in various sensing devices, several new methods have been developed to enhance the speed of PSA. These methods work on the principles of: (1) photoextinction of white light, (2) low-angle forward scattering (Fraunhofer diffraction) of laser light, (3) X-ray absorption, and (4) electrical conductivity. Methods employing photoextinction and X-ray absorption techniques are based on particle settling following Stokes' Law.

The hydrophotometer and the turbidity meter are two instruments that determine PSA by photoextinction of white light. PSA is performed by passing a light beam through a suspension of a dispersed sample and measuring the amount of light transmitted through the sample. Singer et al. (1988) reported that five samples could be analyzed in 1 hr, 40 min using the hydrophotometer. These authors, in a comparison experiment of the Malvern Laser Sizer (to be discussed), Electrozone Particle Counter (to be discussed), the Sedigraph (to be discussed), and the hydrophotometer using

sorted and glacial silt standards, reported that all four methods worked well on the sorted silt standards. However, they reported that the hydrophotometer displayed a slight shift toward the coarser particle sizes in the mean and mode of the particle-size distribution when the glacial silt standards were used. Moreover, they found the hydrophotometer to have the poorest precision among the four instruments for polymodal samples. Samples containing silt-clay mixtures posed the greatest analytical problems due to light dispersion, increased fluid viscosity, and particle-particle interactions. Initial turbulence preventing the settling of the silt and clay particles during sample suspension is also a problem with the hydrophotometer.

Two instruments that use a laser light source for PSA are the Leeds and Northrup Microtrac II¹ and the Malvern Laser Sizer². These instruments operate by passing the laser beam through a continuous flow sample cell and quantifying the emergent beam. As particle size increases, the amount of scattered light increases, but the angle of scattering decreases. A collector lens focuses the scattered light to a solid state photodiode detector array which translates the signal to a proportional volumetric measurement. The range in measured particle sizes is from 700 to 0.7 microns for the Microtrac II Standard Range Analyzer and from 60 to 0.12 microns for the Microtrac II Small Particle Analyzer. The Malvern Laser Sizer employs three different collector lenses, with differing focal lengths, giving a measurable particle size range of 564 to 1.2 microns (Singer et al., 1988). Measurement times can be set from 1 to 999 seconds with a sample size requirement of approximately 0.05 grams to 2 grams for the Microtrac II Small Particle Analyzer and 0.5 to 20 grams for the Microtrac II Standard Range Analyzer while the Malvern Laser Sizer requires about 10 minutes per sample per lens. One notable advantage of these systems is that they are not dependent on particle settling and are thus free from the secondary effects of particle shape, density, optical properties, and settling fluid viscosity. In a comparison study conducted by Singer et al. (1988), the Malvern Laser Sizer displayed a slight shift in the mean and mode of the particle-size distribution toward the coarser fractions and had the poorest resolution when sorted silts and glacial silt standards were tested. An additional problem with the laser system was that different collector lenses do not yield the same particle-size distribution where overlapping measurable particle sizes occurred. The occurrence of this type of problem in the Microtrac II systems is unknown by the author.

The Micromeritics Sedigraph 5100³ combines X-ray absorption and particle settling for the quantification of silt and clay particles. A finely collimated beam of low energy X-rays is passed through a vertically moving sample cell. The sample cell is moved vertically at a controlled rate (to increase the effective settling time) and the emergent X-ray beam is detected and converted into particle-size data. The Sedigraph 5100 has a measurement range of 300 to 0.1 microns equivalent spherical diameter and uses a 50 mL dispersed sample whose precise concentration is not required. The time required to process one sample with particle sizes ranging from 100 to 1 micron is

¹ Leeds and Northrup, Sumneytown Pike, North Wales, PA 19454.

² Malvern Instruments Inc., 10 Southville Road, Southborough, MA 01772

³ Micromeritics Instrument Corporation, One Micromeritics Drive, Norcross, GA 30093-1877

generally around 30 minutes with 100 minutes required for analysis to an endpoint of 0.2 microns using the Sedigraph 5000 (Welch et al., 1979; Berezin and Voronin, 1981). Multiple scans are required for this wide range in particle sizes. In comparison studies with the pipette method, the Sedigraph indicated a finer particle-size distribution and was more precise than the pipette (Welch et al., 1979; Berezin and Voronin, 1981). Several concerns with the Sedigraph system are that instrument down time may be a factor (Schiebe et al., 1981), organic matter sensitivity (Berezin and Voronin, 1981), availability of qualified operators (Schiebe et al., 1981). Although the precise sample concentration is not required, a given concentration range is required for optimal instrument performance. At suspension concentrations of less than 2% by volume, the Sedigraph was found to be both accurate and precise, yet above that point, a shift toward the finer particle sizes was noted (Singer et al., 1988). Stein (1985) reported that soils high in montmorillonite caused problems with the Sedigraph due to the thixotropic properties of montmorillonite.

The Coulter Counter TAI⁴ and the Elzone 180⁵ (also cited in the literature as the Electrozone System) determine PSA by measuring changes in the electrical conductivity as particles pass through a small orifice. As the particles pass through the orifice, the electrolyte is displaced, and a resistance between the electrodes (on both sides of the orifice) is created. This resistance increase is measured by impulse height and discriminantly counted in a multichannel analyzer. A dilute sediment concentration and continual mixing of the sample are required to prevent large particle settling and to eliminate particle coincidence (i.e. particles moving through the orifice at the same time resulting in the counting of a coarser particle size). These instruments are capable of measuring particle sizes from about 1200 to 0.4 microns and require between 10 seconds and 10 minutes per sample. Similar to the laser techniques, the Coulter Counter TAI and Elzone 180 are true sizing instruments that measure particles regardless of their densities, shapes, and the settling liquid viscosity, which are important factors in methods employing particle settling. Several major disadvantages of these systems are (1) multiple orifice sizes are required to cover the range in particle sizes for soils, (2) electrolyte selection, (3) orifice clogging, (4) particle coincidence, the necessity to remove the previously measured coarser particles prior to analysis with each sequentially smaller orifice (Graf, 1979b), and (5) the inability of the system to measure the complete clay fraction. Pennington and Lewis (1979) compared the Coulter Counter TAI with the pipette method using a variety of Idaho soils and found good correlation between the two methods for the silt fractions ($r^2 = 0.80$). For the clay fractions, however, the Coulter Counter results were between one-third to one-half lower than the pipette when clay contents ranged between 29 and 33%.

Reference Method

The reference method for PSA, even in light of the more sophisticated technology now available, is still the separation of sand fractions and the pipette method to quantify silt and clay fractions. Organic matter removal using hydrogen peroxide and sample dispersion using sodium

⁴ Coulter Electronics, Inc., 650 W. 20th Street, Hialeah, FL 33010

⁵ Particle Data, Inc., P.O. Box 265, Elmhurst, IL 60126

hexametaphosphate are recommended. The use of the hydrometer is a viable alternative due to its increased speed in comparison with the pipette and can be used if time is a critical factor. Other previously discussed methods have limitations, such as limited particle size ranges either on the coarse (i.e., can not determine particles up to 2 mm in diameter) or fine (i.e., generally limited to around 0.1 micron and coarser fractions) ends, instrument cost, multiple reading requirements to capture entire particle-size distribution, excessive time requirements for analysis or sample preparation, increased technician skill requirements, and do not have widely accepted and documented standard operating procedures, which decreases their usefulness for PSA. However, with continual improvements in the electronics and X-ray technologies, fast, reliable particle-size determination of the silt and clay fractions may soon be possible.

NOTE: This reference method is for mineral horizon PSA analysis only.

Summary of Method

Organic matter is removed from the sample. The sand fractions are separated from the silt and clay fractions by wet sieving. The silt and clay separates are suspended in water. Aliquots are taken from the suspension under the specified conditions, dried, and then weighed. The resulting gravimetric data allows calculation of the percentages of each particle-size fraction.

Interferences and Shortcomings

The sedimentation cylinder should not be disturbed nor may the temperature vary while the soil in suspension is settling. The use of forceps, finger cots, cotton or vinyl gloves are required to avoid adding weight from moisture, body salts, or body oils when handling weighing bottles, pans, etc. Other sources of error include the deviation of the specific gravity of the soil particles from the assumed 2.65 g/cm³, rising air bubbles after mixing interfering with particle settling, changes in settling velocity due to friction on the sides of the cylinder, and the fact that the particles, especially the clays, are not spherical.

Safety

Use forceps or heat resistant gloves to remove weighing containers from hot ovens. Use waterproof gloves and safety goggles while handling H₂O₂. Follow standard laboratory safety practices when handling reagents and equipment.

Apparatus and Equipment

- Erlenmeyer flask, beaker, or other suitable container, 250 mL or equivalent.
- Beakers, 125 mL, or equivalent.
- Reciprocating shaker, 120 oscillations per minute.

- Sedimentation cylinders, (1 litre graduated cylinders, optional).
- Stirrer, motor-driven.
- Stirrer, hand. Fasten a circular piece of perforated plastic to one end of a rod.
- Shaw pipette rack, or equivalent.
- Pipettes, 25 mL automatic (Lowry with overflow bulb, or equivalent).
- Polyurethane foam pipe-insulation; constant temperature bath ($\pm 1^\circ \text{C}$); or temperature controlled room ($\pm 1^\circ \text{C}$).
- Sieve shaker, 1.25 cm vertical and lateral movement, 500 oscillations per minute, or equivalent. Unit must accommodate a nest of sieves.
- Glass weighing bottles, 90 mL, wide-mouth with screw caps, or equivalent, capable of withstanding intermittent heatings to 110°C .
- Top loading balance (0.01 g sensitivity).
- Electronic analytical balance (0.1 mg sensitivity).
- Set of sieves, square-mesh, woven phosphor-bronze or stainless steel wire cloth; U.S. Series and Tyler Screen Scale equivalent designation as follows:

Nominal Opening (mm)	U.S. No.	Tyler Mesh Size
1.0	18	16
0.5	35	32
0.25	60	60
0.105	140	150
0.053	270	270

- Receiving pan, used with sieves.
- Cover glasses, watch glasses, or equivalent.
- Thermometer, range 10 to 120°C .
- Desiccator and desiccant, P_2O_5 .
- Hot plate (block digester, optional).

Reagents and Consumable Materials

- Hydrogen peroxide (H_2O_2), 30 to 35 percent.
- Sodium carbonate (Na_2CO_3).
- Sodium hexametaphosphate (NaPO_3)₆.
- water—Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- Dispersing agent - Dissolve 35.7 grams (NaPO_3)₆ and 7.94 grams of NaCO_3 per litre of deionized (DI) water.
- Aluminum weighing dishes, or equivalent.

Calibration and Standardization

Calibrate thermometers periodically to ensure that they are measuring temperature accurately. Temperature of the suspensions should not vary more than $\pm 1^\circ \text{C}$.

Procedure

The reference method is divided into four procedures. The first procedure is the removal of organic matter which may be excluded for samples with low organic matter contents at the discretion of the analyst. The remaining three procedures, namely, sample dispersion, sand fraction quantification, and silt and clay quantification, are essential for the determination of PSA.

NOTE: The procedures described are for mineral horizons only.

Procedure One - Removal of Organic Matter

- Step 1 - Weigh $10 \pm 0.01 \text{ g}$ air-dried soil into a tared Erlenmeyer flask, beaker, or equivalent. For soils with low clay contents, it may be necessary to double the amount of soil in order to have a sufficient measurable quantity of clay. Doubling the amount of soil does not require any adjustments to the remaining steps in this procedure.
- Step 2 - Add 50 mL of DI water, followed by 5 mL of H_2O_2 . Cover the flask with a watch glass. If a violent reaction occurs, repeat the H_2O_2 treatment periodically until no more frothing occurs.

Step 3 - Heat the flask to approximately 90° C on an electric hot plate. Add H₂O₂ in 5 mL amounts at 10-15 minute intervals until the organic matter is destroyed as determined visually by the lack of effervescence. Continue heating for approximately 30 minutes to remove excess H₂O₂.

NOTE: Some long-chain organics tend to produce a stable foam which continues to build upon heating. The foam can be controlled by periodic streams of water or methanol from a wash bottle.

NOTE: Do not let samples on hot plate boil dry or certain clays may collapse and may not be successfully dispersed.

Step 4 - Dry the sample overnight in an oven at 105° C, cool the sample in a desiccator, and weigh the H₂O₂-treated sample plus flask to the nearest 0.01 g. Record the weight of the soil + flask. The oven-dry soil weight can then be calculated by subtracting the flask weight from the soil + flask weight. Use the weight of the oven-dry, H₂O₂-treated sample as the soil weight (Treated Sample Weight) for calculating percentages of the particle-size fractions.

Procedure Two - Sample Dispersion and Separation of Sand from Silt and Clay

Step 1 - Add 10 mL of dispersing agent to the flask containing the oven-dry treated sample. Bring the volume to approximately 200 mL using DI water. Stopper the flask and shake overnight on a horizontal reciprocating shaker at 120 oscillations per minute.

Step 2 - Place a 270-mesh sieve on top of the sedimentation cylinder. Wash the dispersed sample onto the sieve with DI water. Avoid using jets of water because they may break the fine mesh of the sieve. Silt, clay, and some very fine sand will pass through the sieve into the cylinder. The sand fractions will remain in the sieve. It is important to wash all particles of less than 0.05 mm diameter through the sieve. Gently tapping the sieve clamp with the side of the hand may facilitate sieving.

NOTE: A clamp and ring stand may be used to hold the sieve in place.

Step 3 - Continue washing the sand until suspension volume in the cylinder is approximately 900 mL.

NOTE: For soils high in silt, wet sieving may be inadequate to separate all the silt from the sand fraction. In this situation, it is recommended that the sample remaining on the sieve be air-dried overnight on the sieve resting on a watch glass to catch any silt passing through during drying. Dry sieve the sample the next day and add any portion passing through the sieve to the sedimentation column prior to proceeding to step 4.

- Step 4 - Remove the sieve from the cylinder. Wash the sand into a tared evaporating dish or original flask with DI water. Dry the sand overnight at 105° C.
- Step 5 - Dilute the silt and clay suspension in the cylinder to 1 litre with DI water. Cover the cylinder with a watch glass.

Procedure Three - Sand Fraction Quantification.

- Step 1 - Wash the sands quantitatively from the sieve (Procedure Two, Step 4) into a beaker or equivalent drying container with DI water and dry the sample overnight at 105° C.
- Step 2 - Remove the sands from the oven and allow to cool to room temperature in a desiccator. Weigh the total dry sand to the nearest 0.01 g and record the weight.
- Step 3 - Stack sieves from the largest mesh opening (1.0 mm) on top to the smallest mesh opening on the bottom. Below the smallest mesh sieve, place the receiving pan.
- Step 4 - Quantitatively transfer the dried sand to the top sieve.
- Step 5 - Cover and secure the stack of sieves in the shaker. Shake the nest of sieves for 3 minutes.
- Step 6 - Quantitatively transfer the sand fractions to tared aluminum weighing dishes (tared to the nearest 0.01 g). Weigh each sand fraction to the nearest 0.01 g and record the weight. The sand fractions contained on top of the sieves are the very coarse, coarse, medium, fine, and very fine listed from top to bottom sieve, respectively.

Procedure Four - Silt and Clay Quantification by Pipetting

NOTE: All pipetting should be performed in a location free from drafts and temperature fluctuations. A temperature-controlled room, constant-temperature water bath, or foam insulation may be used.

NOTE: A blank sample should be prepared with each run to determine the weight contribution of the dispersing agent to the weight of the pipetted aliquot. The blank sample should contain 10 mL of the dispersing agent only, diluted to 1 litre. The blank sample should proceed through all the steps in the following procedure. The net weight of this blank will enter into the final fraction quantification as the RBLANK in the formulae.

- Step 1 - Allow 12 to 24 hours for the suspension temperature to equilibrate to room temperature if temperature control is being maintained by use of either foam insulation, or a constant temperature room, or to equilibrate in the constant temperature water bath.

- Step 2 - Refill sedimentation cylinder to the 1 litre mark with DI water at the same temperature as the analysis to be performed.
- Step 3 - Stir the material in the sedimentation cylinder for 6 minutes with the motor-driven stirrer. Stir 8 minutes if the suspension has been standing for more than 16 hours. If stoppers of adequate size are available, it is preferable to stopper the cylinder and invert. Care must be taken to ensure that the stopper is held tightly in the cylinder. After the cylinder has been inspected to ensure that the fine particles are not adhering to the glass walls or bottom of the cylinder, repeat the inversion procedure at least 6 additional times.
- Step 4 - Remove the stirrer and either (1) cover the cylinder with a length of polyurethane foam pipe-insulation, (2) immerse the cylinder in a constant-temperature water bath, or (3) place the cylinder in a temperature-controlled room.
- Step 5 - Record the temperature of the solution in the cylinder by gently lowering a thermometer, 5 cm into the suspension. Support the thermometer with a clamp to reduce disturbance of the suspension.
- NOTE: A separate cylinder of DI water may be used to monitor the temperature.
- Step 6 - Stir the suspension for 30 seconds with a hand stirrer using an up-and-down motion. Record the time when the stirring is complete. Do not move, stir, or otherwise disturb the cylinder from this point until all pipetting has been completed.
- Step 7 - Determine the appropriate settling time required for the 0.02- to 0.005 mm fraction (medium silt) using Table 1. The resultant pipetting for this fraction will be referred to as Pipette #1 in the calculations.
- Step 8 - About 30 seconds before the sedimentation time has elapsed, slowly lower the Lowry automatic pipet 10 cm into the suspension. (A 25-mL volumetric pipet premarked for a 10-cm depth and clamped firmly in place on a stand may be used.)
- Step 9 - At the appropriate time, slowly fill the pipet (allow for about 12 seconds). Carefully remove the pipet from the suspension.
- Step 10 - Wipe the outside of the pipet clean and empty the contents into a drying container, such as a wide mouth glass weighing bottle or aluminum weighing tins, tared to the nearest 0.1 mg. Rinse the pipette once with DI water and add the rinse water to the contents of the bottle.
- Step 11 - Dry the bottle or tin and contents in an oven overnight at 105° C. Cool in a desiccator over phosphorus pentoxide (P_2O_5). Weigh and record net weight to nearest 0.1 mg.

- Step 12 - Repeat steps 7 through 11 for the 0.005 to 0.002 mm (Pipette #2) and the <0.002 mm (Pipette #3) clay fraction. The <0.002 mm fraction may be pipetted at a time ranging from 5 to 8 hours depending on the temperature and the depth of sampling as listed in Tables 1 and 2. The use of Table 1 is strongly recommended.
- Step 13 - Determination of the fine clay fraction (<0.0002 mm mean diameter) must be performed by centrifuging due to the excessively long time required for sedimentation (up to 39 days) and the influence of Brownian motion on the settling of fine clay particles (Kilmer and Alexander, 1949). Required centrifuging times may be calculated by incorporation of the appropriate gravitational acceleration (g) into Stokes' Law. A more complete presentation of this procedure is presented by Sheldrick (1984).

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. The standard deviation among similar size fraction should be within 3.0 weight % for the sand and silt fractions and within 2.0 weight percent for clay fractions.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known fraction concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

One method blank should be analyzed with each batch of thirty samples. A method blank consists of a 25 mL aliquot of the diluted dispersion solution (10 mL of the hexametaphosphate dispersion solution diluted to 1 L), dried, and weighed. The method blank weight is used in the final calculation of the silt and clay contents.

Quality Control Preparation Sample

A well-characterized soil having a minimum of 5 percent sand, silt, and clay should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Quantified values of the QCCS should be within $\pm 10\%$ of the known quantities within the sample with checking only being performed on the total sand, silt, and clay fractions. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, a new QCPS should be obtained.

Suggested Run Format

MB, Samples 1 to 8, QCPS,
Samples 9 to 16, DUP,
Samples 16 to 25, SRM,
Samples 26 to 30.

where: MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations

NOTE: All quantities of individual size fractions are calculated in weight percent.

$$\text{SAND} = \frac{\left(\text{VERY COARSE SAND} + \text{COARSE SAND} + \text{MEDIUM SAND} + \text{FINE SAND} + \text{VERY FINE SAND} \right)}{\text{Treated Sample Weight}} \times 100$$

$$\text{SILT} = 100\% - (\text{SAND} + \text{CLAY})$$

$$\text{CLAY} = \frac{(\text{Oven-dry Net Weight of Pipette \#3} - \text{RBLANK})}{\text{Treated Sample Weight}} \times \frac{1000}{25} \times 100$$

$$\text{VERY COARSE SAND} = \frac{\text{Oven-dry net weight of very coarse sand}}{\text{Treated Sample Weight}} \times 100$$

$$\text{COARSE SAND} = \frac{(\text{Oven-dry net weight of coarse sand})}{\text{Treated Sample Weight}} \times 100$$

$$\text{MEDIUM SAND} = \frac{(\text{Oven-dry net weight of medium sand})}{\text{Treated Sample Weight}} \times 100$$

$$\text{FINE SAND} = \frac{(\text{Oven-dry net weight of fine sand})}{\text{Treated Sample Weight}} \times 100$$

$$\text{VERY FINE SAND} = \frac{(\text{Oven-dry net weight of very fine sand})}{\text{Treated Sample Weight}} \times 100$$

$$\text{COARSE SILT} = \text{SILT} - (\text{MEDIUM SILT} + \text{FINE SILT})$$

$$\text{MEDIUM SILT} = \frac{(\text{ODNW of Pipette \#1} - \text{ODNW of Pipette \#2})}{\text{Treated Sample Weight}} \times \frac{1000}{25} \times 100$$

$$\text{FINE SILT} = \frac{(\text{ODNW of Pipette \#2} - \text{ODNW of Pipette \#3})}{\text{Treated Sample Weight}} \times \frac{1000}{25} \times 100$$

where: ODNW = oven-dry net weight.

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Soil pH

Introduction

Soil pH is one of the most indicative chemical measurements in soil. Soil pH is a measure of the hydrogen ion activity in the soil solution and can, therefore, be considered as the intensity factor for soil acidity. The importance of soil pH is that it is integral to many other soil properties such as the solubility of compounds, the availability of plant nutrients, the relative bonding of ions to exchange sites, and the activity of various soil microorganisms. It is integral in the sense that soil pH is both a symptom and a cause of the many reactions that occur within soil.

Soil pH is recommended as a parameter for terrestrial monitoring programs for several reasons. One of the effects of acid deposition on soil is soil acidification. Decreases in soil pH resulting from soil acidification will reflect the overall decline in base saturation and an increase in exchangeable acidity (Bache, 1980). Soil pH can be measured with relatively high precision by present analytical methods. The spatial variability of soil pH is also lower than a majority of other soil properties (Arp and Krause, 1984). The temporal variability in this parameter can also be minimized through the selection of appropriate methods.

Review of Methods

The value obtained for pH in a soil is very dependent upon the method used for its measurement. Several factors that influence the measured pH value include the soil/solution ratio, electrolyte concentration, CO₂ content of the soil/solution, temperature, nature and placement of the electrodes, and treatment of a soil prior to measurement. Common soil/solution ratios on a weight basis include 1:1, 1:2, 1:4, 1:5, and 1:10, and saturated pastes (McLean, 1982). Common electrolytes used in the measurement of soil pH include deionized water, 0.01 M CaCl₂, 1 N KCl and 0.002 M CaCl₂ (Cappo et al., 1987). The higher the electrolyte concentration, the larger the amount of exchangeable acidity displaced from soil exchange sites with the subsequent lowering of the pH of the soil solution. The displacement of hydrogen ions by the cations of the salts can also be accompanied by the displacement of exchangeable aluminum, which upon hydrolysis, increases the H⁺ ions in the soil solution. The CO₂ content of soil influences the measured pH through the formation of carbonic acid as it dissolves in water. One reason for stirring and allowing a soil to stand for a period of time prior to measurement is to allow the soil CO₂ content to come into equilibrium with CO₂ in the air. Commonly used times are 30 minutes or 60 minutes.

The temperature at the time of measurement affects not only the activity of the H⁺ ion, but the calibration of the instrument. The placement of the electrodes in relationship to the sediment or the supernatant can have a marked effect. This effect has been attributed to the liquid junction potential at the calomel electrode and to differences in H⁺ concentration with distance from soil particles (McLean, 1985). The prior treatment of a soil can affect the pH measurement. The method of drying and storage conditions of the soil can be important. The pH of air-dried soils are generally lower than field moist samples.

Reference Method

The reference solutions for mixing with soil for the soil pH measurement are 0.01 M CaCl₂ and deionized water. Measurement in these two solutions has been recommended by the Canadian Society of Soil Science (McKeague, 1976), the American Society of Soil Science (McLean, 1985), and the U.S. Environmental Protection Agency (Cappo et al. 1986), and are widely used by soil testing laboratories. Measurement of soil pH in 1 N KCl is not recommended since the solution is not

representative of electrolyte concentrations found in most natural soils. The high electrolyte concentration of the KCl solution will result in excessive amounts of exchangeable acidity being displaced by this electrolyte. Measurement of soil pH in 0.01 M CaCl_2 has the advantage of minimizing temporal variability in soil pH due to seasonal salt fluctuations, is independent of soil/solution ratios, and it minimizes potential problems with liquid junction potentials by flocculating soil suspensions. Another standard solution for measuring soil pH is deionized water for which numerous amounts of data are available for comparison. These two measures of soil pH are highly correlated. If both pH values are measured, inaccurate data points can be identified as not being internally consistent.

The reference soil:solution (by weight) ratios are 1:1 for mineral soils and 1:5 for organic soils. The saturated paste technique for measuring soil pH is not recommended due to poorer precision of measurement and potential problems with liquid junction potentials. Laboratory methods are recommended over field methods due to the difficulty of obtaining reproducible results in the field. Improved control over temperature, sample mixing, and subsampling are possible in the laboratory whereas this control is lost during field pH measurements.

A standard KCl combination electrode with a sleeve is preferred for measurement over the double electrode system. The combination electrodes are simpler to handle and maintain, can be used on smaller samples, have a constant distance between the junction and the glass electrodes, and are less prone to potential stoppage of flow of electrolyte from the calomel electrode.

A period of one hour is suggested for equilibration time with regular stirrings at 15 minute intervals to allow the soil to come to equilibrium with the CO_2 in the air in the laboratory. The method presented is an adaptation of the method presented by Capps et al. (1986).

Summary of Method

Two suspensions of each soil sample are prepared, one in DI water, and one in 0.01 M CaCl_2 . The pH of each suspension is measured with a pH meter and a combination electrode.

Interferences and Shortcomings

Soils high in salts, especially sodium (Na^+) salts, may interfere with the pH reading and the electrode response time.

Clays may clog the KCl junction and may slow the electrode response time. Thorough cleaning of the electrode between samples can help avoid this problem.

Wiping the electrode dry with cloth, laboratory tissue, or similar materials or removing the electrode from solution when the meter is not on standby may cause electrode polarization and, therefore, should be avoided.

The initial pH of a non-alkaline soil will usually be as much as 0.5 pH unit greater than the pH taken after the sample has set for 30 minutes or longer.

The pH will vary as much as 1.0 pH unit between the supernatant and soil sediment. Always place the electrode junction at the same distance above the surface of the soil to maintain uniformity in pH readings.

Safety

No specific hazards are associated with this procedure or with the required reagents. Normal laboratory safety practices are to be observed. Protective clothing and safety glasses should be worn, especially when handling concentrated HCl and dry $\text{Ca}(\text{OH})_2$ to prepare reagents.

Apparatus and Equipment

- digital pH/mV meter, capable of measuring pH to ± 0.01 pH unit and potential to ± 1 mV and temperature to $\pm 0.5^\circ \text{C}$. The meter must also have automatic temperature compensation capability.
- pH and reference electrodes, high quality, low-sodium glass. Gel-Type reference electrodes must not be used. A combination electrode is strongly recommended, and the procedure is written assuming that one is used. The Orion Ross combination pH electrode, or equivalent, with a retractable sleeve junction is preferred. At least two electrodes, one a backup, should be available to the analytical laboratory.
- beakers, plastic or paper containers, 50 mL
- glass stirring rods or disposable stirrers, one per sample.

Reagents and Consumable Materials

- pH buffers of pH = 4, pH = 7, and pH = 10, for electrode calibration.
- buffer of pH 4.0 as a quality control check standard (QCCS). The QCCS can be purchased, or it can be prepared from 0.05 M potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$ or KHP). This buffer must be from a different container or lot than the standards used for electrode calibration.

Dry KHP for 2 hours at 110°C , cool to room temperature in a desiccator. Weigh 10.21 g of KHP, dissolve it in DI water, and dilute the solution to 1.000 L. To preserve the KHP solution, add 1.0 mL of chloroform or one crystal (about 10 mm in diameter) of thymol per litre of the buffer solution. This solution has the following pH values at the temperatures given:

<u>T($^\circ\text{C}$)</u>	<u>pH</u>
15	3.999
20	4.002
25	4.008
30	4.015

- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

- stock calcium chloride solution (CaCl_2 , 1.0 M)--Dissolve 55.493 g of anhydrous CaCl_2 in DI water and dilute to 500 mL.
- calcium chloride 0.01 M CaCl_2 --Dilute 20 mL of stock 1.0 M CaCl_2 to 2.000 L with DI water. If the pH of this solution is not between 5 and 6.5, adjust the pH by addition of dilute $\text{Ca}(\text{OH})_2$ or HCl, as needed. Verify the concentration of the CaCl_2 solution by measuring the electrical conductivity. The specific conductivity should be 2.32 ± 0.08 mmho/cm at 25°C . If it is not, prepare fresh solution.
- calcium hydroxide ($\text{Ca}(\text{OH})_2$)--Dissolve 0.185 g $\text{Ca}(\text{OH})_2$ in 1 L of DI water.
- hydrochloric acid (HCl)--Dilute 1 mL concentrated HCl to 1 L with DI water.
- potassium chloride (KCl, 3 M)--Dissolve 224 g of KCl in DI water and dilute to 1 L.
- potassium chloride (KCl, 0.1 M)--Dissolve 74.5 g of KCl in DI water and dilute to 1 L.

Calibration and Standardization

For storage and readings, the electrode need only be immersed to cover the liquid junction of the reference electrode (typically about 2.5 cm).

Rinse electrode with DI water between each sample and each buffer to prevent solution carry-over. Do not rub or blot electrode dry because this may produce a static electric charge and, thereby, polarize the electrode.

To prepare the pH electrode for use, move the band covering the fill hole and fill the reference reservoir to the hole with 3 M KCl filling solution. Allow 5 minutes for the ceramic frit to become wet with filling solution before immersing the electrode in sample or buffer. The retractable sleeve junction allows easy cleaning of clay particles and other insoluble compounds that clog the junction and, thereby, produce drift and slow response.

Each analyst must be thoroughly acquainted with the procedure and familiar with all instrument functions. Read and follow all operating and start-up procedures for the pH meter. Leave the instrument on standby and verify that the combination electrode is connected and that the level of reference filling solution is at least 3 cm above the sample surface. Check the temperature calibration by measuring room temperature of a solution with the electrode and meter and with a thermometer.

Calibrate the electrode at a minimum of two points that bracket the expected pH and that are three pH units or more apart. Use standardized buffers of pH 4, 7, and 10 for samples in the expected pH range.

Stir pH 4.0 buffer solution for 30 seconds, read the pH after equilibration, and adjust the meter, if necessary. Perform the step again, using the pH 7.0 buffer. Repeat measurements and adjustments until readings for both buffer solutions are within 0.1 pH units of the respective true buffer values. Repeat the process substituting a pH 10.0 buffer in place of the pH 4.0 buffer for soils of pH greater than 7.0.

Procedure

- Step 1 - Prepare two suspensions of each soil sample, one in DI water and one in 0.01 M CaCl_2 , using soil-to-solution ratios of 1:1 for mineral horizons and 1:5 for organic horizons. For mineral horizons, add 20 mL of the appropriate solution to 20.00 g soil. For organic horizons, add 25 mL solution to 5.00 g soil.
- Step 2 - Allow soil to absorb solution without stirring, then thoroughly stir the soil-solution mixture for 10 seconds with a glass stirring rod or disposable stirrer. Stir again for 10 seconds after 15, 30, 45, and 60 minutes.
- Step 3 - After the final stirring, allow the suspension to settle for 1 minute. Place the pH electrode in the supernatant of the soil suspension.
- For mineral soils, the electrode junction should be below the solution surface and above the soil-solution interface.
- Some organic soils swell, so there is no free water available. As long as the electrode junction is below the surface of the organic material, an acceptable, repeatable reading is generally attained. If the reading is not stable, add enough solution to cover the electrode junction. When the reading is stable, record pH to the nearest 0.01 pH unit.
- Step 4 - Report the pH of the soil:DI water suspension and the soil:0.01 M CaCl_2 suspension for each sample.
- Step 5 - After measurements are completed, store the electrode in 0.1 M KCl manufactured storage solution. Do not let the sensing element and reference junction dry out. The level of the storage solution should be 1 inch below the filling solution level to prevent influx of the storage solution. Periodically check that the electrode reservoir is full of filling solution.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples for each of the following solutions: DI water and 0.01 M CaCl_2 . To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on absolute difference between the samples at an acceptance limit for the difference of ≤ 0.1 pH unit.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within ± 0.1 pH unit of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed.

Quality Control Check Standard

A quality control check standard (QCCS), a pH 4.0 standard that is either from a different purchased lot or created at a different time, should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. Values for the QCCS should be 4.00 ± 0.05 pH units.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, Samples 1 to 10,
QCCS, Samples 11 to 20,
QCCS, Samples 20 to 30, QCPS, DUP, QCCS.

where: QCCS = quality control check standard
QCPS = quality control preparation sample
DUP = duplicate sample

NOTE: The run format presented is per solution (i.e., DI water or 0.01 M CaCl_2).

Calculations and Reporting

No calculations are required to obtain pH values in pH units.

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Organic Carbon

Introduction

In soil, carbon (C) occurs in mineral and organic forms. In acid soils, where carbonate minerals are generally lacking, most C occurs in organic matter. Organic matter consists of plant, animal, and microbial remains in various stages of decomposition plus their highly-altered derivatives. The latter are collectively referred to as humic matter or humus. Humus maintains the physical condition, improves moisture holding capacity, serves as an energy source for soil organisms, and increases the nutrient holding capacity (particularly of nitrogen and phosphorus) of the soil. Further, quantification of carbon, in conjunction with total N and S, provides insight about the potential for uptake or release of N and/or S by the soil organic matter due to microbial activity (Blume et al., 1990). Therefore, the measurement of organic carbon in the soil is suggested for terrestrial monitoring programs, such as LRTAP.

In addition to humus (and considered "organic" because of their origin) are several highly-condensed, nearly-elemental forms of C, such as charcoal and graphite. These may form a significant portion of the total organic C present in soils with a history of frequent fires. In contrast to humus, these highly-condensed forms are relatively inert. Thus, it is desirable to discriminate not only between mineral and organic C, but between the readily oxidizable and inert organic C forms.

The mineral and inert carbon forms are indistinguishable from the organic carbon forms using the total carbon procedure presented in Chapter 9. Thus, this method is presented to address the determination of organic C in soils where carbonates or noticeable quantities of charcoal are present. In most acid soils, however, the determination of carbon can be made using either this method or the total carbon method presented in Chapter 9.

Review of Methods

There are a number of approaches to the determination of organic C in soil: (1) as the difference between total (i.e. all forms) C and the mineral (chiefly carbonate) forms present, (2) by determining total C after removing inorganic C by acid treatment, (3) by dry combustion (in a muffle furnace at moderate temperature) with the organic content expressed as weight "loss-on-ignition", and (4) by organic matter reduction of $\text{Cr}_2\text{O}_7^{2-}$ and the subsequent titrimetric determination of the unreacted $\text{Cr}_2\text{O}_7^{2-}$ by ferrous sulphate. The first three methods estimate total organic C, without discrimination between humus and elementary C. Further, the first two methods require specialized equipment. Loss-on-ignition, while straightforward and acceptable for descriptive purposes, may provide over-estimate of reactive organic materials in soils of high charcoal content, or in certain clay soils where weight loss may be associated with loss of water or hydroxyl groups.

Schollenberger (1927) introduced a rapid titrimetric method whereby soil organic matter is oxidized by a saturated solution of potassium dichromate in concentrated sulphuric acid with application of heat. The unreduced chromic acid is then back-titrated with ferrous ammonium sulphate solution. Walkley and Black (1934) and later Walkley (1947) modified this by omitting the heating step, utilizing only the heat of dilution of H_2SO_4 with water. The procedure is about 75% effective in recovering organic C with the exact figure varying somewhat with the soil. Walkley and Black (1934) found a mean recovery of 76% for a range of British and foreign soils, necessitating a multiplying factor of 1.32 to yield a result equivalent to organic C by combustion methods. Allison (1960) and others, however, suggested a much broader range varying with soil group. In the absence of a specific value, however, the original Walkley-Black (1934) value (76%) is frequently used. The original Walkley-Black procedure has been modified by a number of authors. Readily available manuals, all giving variants of the "Walkley-Black" method include: Metson (1956), Jackson (1958), Allison (1965), McKeague (1978), Nelson and Sommers (1982), Heffernan (1985), and Kalra and Maynard (1991).

Reference Method

The following procedure is that presented by Walkley (1947) with minor wording changes only. Ferrous ammonium sulphate $[\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$ may be substituted for ferrous sulphate; ortho-phenanthroline ferrous complex ("Ferroin"), instead of diphenylamine, may be used as the indicator.

Summary of Method

A sample is oxidized with 1 N potassium dichromate and concentrated sulphuric acid. After 20 to 30 minutes, the reaction is halted by dilution with water. Phosphoric acid and diphenylamine indicator are added to the solution. The excess dichromate is potentiometrically back-titrated with ferrous sulphate. A blank is carried throughout the procedure to standardize the ferrous sulfate solution. Percent organic C is reported on an oven-dry soil basis.

Interferences and Shortcomings

Chloride (Cl^-), if present in substantial quantity, interferes with the Walkley-Black procedure through the formation of chromyl chloride (CrO_2Cl_2). Where problematic, the soil can be leached free of Cl^- before analysis (Schollenberger, 1945). Alternatively, the Cl^- can be precipitated as AgCl by the use of H_2SO_4 containing 25 g Ag_2SO_4 per L in the digestion. A correction factor equal to one-twelfth of the Cl^- content may also be used in soils with a $\text{Cl}:\text{C}$ ratio up to 5:1 (Walkley 1935, 1947; Jackson, 1958; Nelson and Sommers, 1982).

Nitrate (NO_3^-) interferes only if the ratio of C to NO_3^- is less than 20:1 (Heffernan, 1985; Kalra and Maynard, 1991).

While higher oxides of manganese (principally MnO_2 , but also Mn_2O_3 and Mn_3O_4) present a potential interference, it would appear that only freshly-precipitated MnO_2 is capable of competing with Cr_2O_7 for oxidizable substances. Thus, MnO_2 interference can be generally discounted in most soils (Walkley, 1947; Allison, 1965; Nelson and Sommers, 1982). If, however, large quantities of reactive Mn oxides were to be encountered their effect can be nullified by pretreatment with FeSO_4 (Walkley, 1947; Jackson, 1958).

Ferrous iron (Fe^{2+}), if present, is oxidized to Fe^{3+} by $\text{Cr}_2\text{O}_7^{2-}$ leading to a high result. However, thorough air-drying of even highly-reduced soils before analysis is sufficient to convert the Fe^{2+} to Fe^{3+} and negate the effect (Walkley, 1947; Jackson, 1958; Nelson and Sommers, 1982). The amount of Fe^{2+} present in well-aerated soils is so small that its effect can be neglected. Iron or steel equipment should be avoided because of the possibility of introducing reducing material in the form of metallic Fe (Jackson, 1958; Allison, 1965; Nelson and Sommers, 1982; Heffernan, 1985).

Safety

All operations should be carried out in well-ventilated conditions. Protective clothing including eye protection should be worn at all times, and especially when handling concentrated acids. Special care must be taken when adding water to concentrated H_2SO_4 . Diphenylamine has carcinogenic properties and should be handled with extreme caution. Gloves and a respirator should be worn when preparing the indicator solution.

Apparatus and Equipment

- flasks, Erlenmeyer, 500 mL.
- dispenser, capable of accurately dispensing 25 mL.
- shaker, reciprocating, Eberbach or equivalent.
- burette, 100 mL.
- balance, accurate to 0.001 g.

Reagents and Consumable Materials

- sulphuric acid, H_2SO_4 , concentrated, reagent grade (not less than 96%).
- phosphoric acid, H_3PO_4 , concentrated, reagent grade.

- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- diphenylamine indicator--To 0.5 g of indicator powder, add 20 mL water and 100 mL conc. H_2SO_4 .
- potassium dichromate solution, $\text{K}_2\text{Cr}_2\text{O}_7$, 1.0 N--In a one litre volumetric flask, dissolve 49.04 g $\text{K}_2\text{Cr}_2\text{O}_7$ in DI water. Dilute to volume with DI water.
- ferrous sulphate solution, 1 N--In a one litre volumetric flask, dissolve 278.0 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in DI water. Add 15 mL conc. H_2SO_4 . Dilute to volume with DI water.

Calibration and Standardization

Standardization of the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ is required for the accurate determination of organic carbon contents. Standardization is performed using the method blank. Normality of the standardized ferrous sulphate solution is calculated using the formula presented in the "Calculations and Reporting" section. Calibration standards should be prepared in the extraction solution.

Procedure

- Step 1 - Grind sufficient soil for convenient sampling to pass a 0.5-mm screen, avoiding mortars of steel or iron. Transfer a weighed quantity, not exceeding 10 g and containing about 10 to 25 mg of organic carbon, to a 500 mL Erlenmeyer flask.
- Step 2 - Add 10 mL of 1.0 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution and swirl flask gently to disperse soil in the solution.
- Step 3 - Rapidly add 20 mL of concentrated H_2SO_4 .
- Step 4 - Immediately shake the flask once or twice and allow it to stand for 20 to 30 minutes.
- Step 5 - Add 200 to 300 mL DI water, 10 mL of concentrated H_3PO_4 , and 1 mL diphenylamine indicator solution.
- Step 6 - Run in 1.0 N $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution from a burette until the solution is purple or blue.
- Step 7 - Continue adding 1.0 N $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution in portions of about 0.5 mL until the colour flashes to green, which it does with little or no warning.
- Step 8 - Add 0.5 mL 1.0 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution and complete the titration by adding 1.0 N $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution dropwise until the last trace of blue disappears. Record volume of titrant used.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

One method blank, carried through the extraction procedure, are analyzed with each batch of thirty samples to determine the exact normality of the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
 QCCS, Samples 9 to 16, DUP,
 QCCS, Samples 16 to 25, SRM,
 QCCS, Samples 26 to 30, QCCS.

where: QCCS = quality control check standard
 MB = method blank
 QCPS = quality control preparation sample
 DUP = duplicate sample
 SRM = standard reference material

Calculations and Reporting

The following calculations assume that a total of 10.5 mL $K_2Cr_2O_7$ solution is used and that the recovery is greater than 75%. If a soil-specific recovery other than 75% is identified, then that factor should be used. If the results are to be expressed in terms of "readily oxidizable organic C", the recovery factor is omitted (Jackson 1958).

1 mL 1.0 N $K_2Cr_2O_7$ is equivalent to 0.003 g C.

- (1) Determine exact normality of $FeSO_4 \cdot 7H_2O$ solution from the method blank as follows:

$$\text{Normality of } FeSO_4 \cdot 7H_2O = \frac{10.5}{\text{mL } FeSO_4 \cdot 7H_2O \text{ used}}$$

- (2) Determine volume of $K_2Cr_2O_7$ reduced as follows:

$$\text{mL } K_2Cr_2O_7 \text{ Reduced} = \text{mL } 1 \text{ N } K_2Cr_2O_7 \text{ used} - (\text{mL } FeSO_4 \cdot 7H_2O \times \text{N } FeSO_4 \cdot 7H_2O)$$

- (3) Calculate "total organic C" (%) as follows (the calculation assumes that the oxidation is 76% efficient)

$$\text{Total Organic C \%} = \frac{(\text{mL } K_2Cr_2O_7 \text{ reduced}) \times 0.395}{\text{weight (g)}}$$

- (4) Alternatively, assuming the soil organic matter:organic C ratio to be 1.724, the results may be expressed as "total organic matter" (OM)

$$\text{Total OM \%} = \frac{(\text{mL K}_2\text{Cr}_2\text{O}_7 \text{ reduced}) \times 0.681}{\text{weight (g)}}$$

- (5) Alternatively, the results may be expressed as "readily oxidizable organic C" (the calculation omits recovery factor)

$$\text{Readily Oxidizable Organic C \%} = \frac{(\text{mL K}_2\text{Cr}_2\text{O}_7 \text{ reduced}) \times 0.3}{\text{weight (g)}}$$

- (6) Alternatively, the results may be expressed as "readily oxidizable organic matter".

$$\text{Readily Oxidizable OM \%} = \frac{(\text{mL K}_2\text{Cr}_2\text{O}_7 \text{ reduced}) \times 0.517}{\text{weight (g)}}$$

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- Walkley, A., and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.

Total Carbon

Introduction

In soil, carbon occurs in both mineral and organic forms. In soils affected by acidic deposition, carbonate minerals (limestone and dolomite) are generally lacking and thus, most C occurs in organic matter. As a result of this lack of inorganic carbon forms, total carbon analyses in eastern forest soils affected by acidic deposition are often referred to as total organic carbon (TOC) measurements.

Organic carbon forms consist of plant, animal, and microbial remains in various stages of decomposition plus their highly-altered derivatives. The latter are collectively referred to as humic matter or humus. Humus maintains the physical condition, improves moisture holding capacity, serves as an energy source for soil organisms, and increases the nutrient holding capacity (particularly of nitrogen and phosphorus) of the soil. In addition to humus (and considered "organic" because of their origin) are several highly-condensed, nearly-elemental forms of C, such as charcoal and graphite. These nearly-elemental forms may compose a significant portion of the total organic C present in soils with a history of frequent fires. In contrast to humus, these highly-condensed forms are relatively inert in the soil.

Total carbon is the sum of all inorganic and organic forms of carbon in the soil (Nelson and Sommers, 1982). Quantification of total C, in conjunction with total N and S, provides insight about the potential for uptake or release of N and/or S by the soil organic matter due to microbial activity (Blume et al., 1990). The carbon content of a soil affects sulphate adsorption and cation exchange properties of the soil, and TC content is sometimes used for certain soil taxonomic classifications such as the determination of a mollic (and thus, Mollisols) and histic epipedons (Soil Survey Staff, 1975). Therefore, the measurement of total carbon in the soil is suggested for terrestrial monitoring programs, such as LRTAP.

Review of Methods

Total carbon determinations for soil involve the conversion of all forms of C to CO₂ by either dry combustion or wet digestion techniques. The evolved CO₂ is then quantified by gravimetric, titrimetric, volumetric, manometric, spectrophotometric, or gas chromatographic techniques (Nelson and Sommers, 1982).

Direct analysis of the sample may be done by combustion of the sample at elevated temperatures (generally at 1000° C or higher; 1300° C is optimal for the completion destruction of calcium carbonate) and measuring the liberated CO₂ by an infrared or thermal conductivity spectroscopy. Combustion is performed in a resistance furnace or an induction furnace in the presence of a stream of oxygen or CO₂-free air. Various catalyst or accelerators may be used to

ensure complete combustion of all carbon forms. Dry combustion procedures using either high-temperature (approximately 1500° C) or induction furnaces are most commonly found in commercially-available automated total C analyzers (Nelson and Sommers, 1982). Examples of instruments that combust the sample include the LECO CHN analyzers and Carla-Erba C and N analyzers. These methods require little or no sample preparation for the determination of total C.

The wet digestion analysis of soils for total C by chromic acid digestion has long been a standard method giving good agreement with the dry combustion techniques (Nelson and Sommers, 1982). Schollenberger (1927) introduced a rapid titrimetric method whereby soil organic matter is oxidized by a saturated solution of potassium dichromate in concentrated sulphuric acid with application of heat. The unreduced chromic acid is then back-titrated with ferrous ammonium sulphate solution. Walkley and Black (1934) and later Walkley (1947) modified this by omitting the heating step, utilizing only the heat of dilution of H₂SO₄ with water. The procedure is about three-quarters effective in recovering organic C with the exact figure varying somewhat with the soil. Walkley and Black (1934) found a mean recovery of 76% for a range of British and foreign soils, necessitating a multiplying factor of 1.32 to yield a result equivalent to organic C by combustion methods. Allison (1960) and others, however, suggested a much broader range varying with soil group. Alternately, wet combustion may be carried out in a Van-Slyke-Neil apparatus and evolved CO₂ estimated by manometric procedures (Bremner, 1949; Nelson and Sommers, 1982). However, it should be noted that these methods are only comparable to the dry combustion techniques in soils in which no carbonates or inert forms of carbon (e.g., charcoal or graphite) are present.

Reference Method

The reference method for total C in soils is dry combustion in a carbon analyzer with either infrared or thermal conductivity detection. The analysis of total C employing this technique has been chosen as a reference method because it is widely used in North America and has been used successfully by some laboratories for both soil and foliage samples.

Summary of Method

A soil sample is oxidized at temperatures greater than 1000° C (1300° C preferred) with catalysts as specified by the instrument manufacturer. The evolved CO₂ is then quantified by either infrared or thermal conductivity detection. Percent total C is reported on an oven-dry soil basis. Depending on the type of instrument present in the laboratory, total N and H can also be simultaneously quantified from the same sample aliquot using this technique.

Interferences and Shortcomings

Although moisture can interfere with certain carbon-hydrogen-nitrogen (CHN) analyses by producing large responses for total H, this interference can be eliminated in elemental analyses with moisture traps. Drying can cause losses of volatile organic materials containing C and N, and

decomposition and loss of certain carbonates and ammonium salts. Samples may be freeze-dried to minimize these losses if approved by the quality assurance (QA) manager.

Ambient N_2 and CO_2 not associated with the sample present possible gaseous interferences. Care must be taken with the blank to hold N_2 and CO_2 below the required instrument detection limit. The use of high purity carrier gas or helium traps helps reduce CO_2 contamination.

Soil residue can accumulate at the top of the combustion column. The column should be cleaned if sufficient residue accumulates that analytical results are affected.

Safety

Normal safety precautions should be taken when using high-temperature combustion furnaces.

Protective clothing and safety glasses should be worn when handling reagents. Heat resistant gloves may be needed when placing samples in the furnace. The furnace must be adequately vented and protected from human contact and combustible materials. Gas cylinders should be bolted or chained in an upright position.

Apparatus and Equipment

- carbon analyzer with infrared detector, Carlo-Erba model 1500, or equivalent.
- balance, accurate to 0.001 g.
- balance calibration weights, 3-5 weights covering expected range.

Reagents and Consumable Materials

- oxygen, high purity.
- compressed air, if needed.
- catalysts and combustion accelerators, vanadium pentoxide or as recommended by instrument manufacturer.
- absorbents, as needed.
- vials, crucibles, boats, or tin sample capsules, as required by instrument.

Calibration and Standardization

Set up the instrument according to the instrument manufacturer's instructions regarding calibration and standardization. In general, the instrument should be calibrated at least once a day or once per batch of samples, whichever is more frequent. Use either NIST (formerly NBS) reference materials or standards supplied by the manufacturer and approved by the laboratory or QA manager. The concentration range of the standards must be representative of the C concentrations expected in the soil samples. A minimum of a two-point calibration curve should be used. Use of a NIST standard reference material as an initial calibration check is highly recommended.

Procedure

- Step 1 - Weigh out 100 mg of air-dried mineral soil or 20 mg of organic soils into appropriate sample holder. Record sample weight.
- Step 2 - Perform sample analysis following manufacturer's instructions.
- Step 3 - Take carbon measurement reading (in counts).

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, containing all accelerators or catalysts used in routine sample analysis in their appropriate proportions, should be carried through the combustion procedure with each batch of samples to measure potential contamination. Method blanks should be run at the

beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

Each laboratory should consult the manufacturer's instruction manual for the determination of the appropriate formulae to be used in the calculation of total C.

For some total C analyzers, % C can be read directly from the instrument. The sample weight, as different from the standard, is taken into account on some instruments.

Results are reported to two significant figures. Results are read to the nearest 0.01%. Results should be blank and moisture corrected.

In general, the total organic carbon (assuming the lack of or negligible quantities inorganic carbonates in the soil) may be calculated as follows:

$$C \text{ (wt \%)} = \frac{(\text{instrument reading} - \text{blank}) \times \text{instrument factor}}{(\text{sample weight (g)} \times \text{moisture correction})}$$

NOTE: The moisture correction factor is as follows:

$$[(1 - \text{moisture content in \%}) + (100 + \text{moisture content in \%})]$$

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Total Nitrogen

Introduction

Nitrogen is one of the five major constituents of living matter. As such, its form and concentration are of major interest when dealing with the growth of terrestrial and aquatic organisms. In nature, nitrogen is found in a number of forms, all of which are interconvertible given the correct chemical and microbiological conditions. Soil N accounts for only a small fraction of N in the lithosphere, and of this fraction, only a very small proportion is directly available to plants since greater than 90% of the N is in the organic form. Plants obtain most of their N from inorganic ions, NO_3^- and NH_4^+ , that comprise less than 1% of the total N.

Atmospheric deposition of NO_x can directly affect the total soil N concentrations. Further, soil N content changes may be brought about by indirect effects to the forest ecosystem, such as a result of acid deposition. Therefore, the measurement of total soil N is suggested for long-term terrestrial monitoring programs, such as LRTAP. Nitrate and ammonium levels in soils are usually negligible and the spatial and temporal variability associated with these parameters is much greater than for total N.

Review of Methods

Total N in soil is usually measured by either a wet oxidation (Kjeldahl method) or dry oxidation (Dumas method) procedure (Bremner and Mulvaney, 1982). Considerable modifications have been proposed for these methods (e.g., Nelson and Sommers, 1980). More recently, near infrared reflectance spectroscopy (NIRS) and LECO combustion have been used as alternatives to the conventional chemical analysis for total N determination. However, these are not in widespread use and involve expensive equipment. Thus, the chemical wet oxidation techniques remain the most frequently used.

The principle of the Kjeldahl and Dumas techniques have been thoroughly discussed in several reviews (Bremner, 1965; Nelson and Sommers, 1980; Bremner and Mulvaney, 1982). The Dumas methods do not quantitatively recover many nitrogenous compounds (e.g., heterocyclic compounds) and the automated N analyzer based on the Dumas method has not been widely used (Bremner and Mulvaney, 1982). The Kjeldahl procedure is more commonly used and modifications of the original Kjeldahl methods have extended the scope of the procedure. The total Kjeldahl N method involves the digestion of the sample with H_2SO_4 to convert organic N to NH_4^+ -N followed by distillation of the digest with strong alkali to liberate NH_3 . Various modifications to the distillation and measurement of the liberated NH_3 have been proposed. In addition, various methods for the direct measurement of NH_4^+ in the Kjeldahl digests have also been used. Highly refractory organic N compounds or compounds containing N-N or N-O linkages are not completely recovered by the Kjeldahl digestion (Bremner and Mulvaney, 1982). However, in most soils, little of the available N is in the refractory form. Similarly, NO_3 -N and NO_2 -N are not adequately recovered but are not usually present in significant amounts in undisturbed forest soils.

Reference Method

The reference method uses the Kjeldahl acid digestion in the presence of a catalyst which converts organic nitrogen to inorganic ammonium. The measurement of N is done using alkaline distillation to liberate NH_3 which is measured by acid titration. The digestion is carried out either on a hot plate or in an aluminum digestion block.

Summary of Method

Soil samples are digested by the regular Kjeldahl technique using a K_2SO_4 - $CuSO_4$ catalyst in an aluminum block digester with internal heaters and temperature control. Determination of NH_4^+ -N is done by an automated system (Tecator Kjeltac 1030) determining NH_3 liberated by distillation of the digest with 40% NaOH. The NH_3 is absorbed in unstandardized H_3BO_3 and ammonium borate is formed. The borate is titrated back to H_3BO_3 using a standard strong acid (HCl).

Interferences

After digestion, samples must not be allowed to cool in the digestion block as NH_3 will be lost from the $(NH_4)_2SO_4$ formed by the digestion.

Safety

Protective clothing and safety glasses should be worn when handling strong acids. The digestion blocks should be located in the fume hood and, if possible, the temperature controller should be located outside the fume hood. The equipment should not be left unattended. The preparation of the NaOH should also be done in the fume hood. The NaOH pellets should be added very slowly to the water and in very small portions due to the intense exothermic reaction that occurs.

Apparatus and Equipment

- digestion block, 20 place, Tecator System 20 1050 or equivalent, with programmable temperature controller.
- distillation and titration apparatus, Kjeltac Auto 1030 Analyzer or equivalent.
- glass digestion tubes (295 mm x 40 mm), 250 mL to fit block, appropriate to sample and solution volume used.
- balance, accurate to 0.001 g.

Reagents and Consumable Materials

- sulphuric acid, H_2SO_4 , concentrated, reagent grade (96%).
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- catalyst, Kjeltab tablets or equivalent. Each tablet contains 3.5 g K_2SO_4 and 0.4 g $CuSO_4$.
- hydrochloric acid, HCl, standard acid 0.01 M.
- boric acid, H_3BO_3 , reagent grade powder.
- ammonium chloride, NH_4Cl , reagent grade powder.
- methanol, reagent grade.

- sodium hydroxide, NaOH, reagent grade pellets.
- sodium hydroxide solution, 40%--Dissolve 10 kg of NaOH in 15 L DI water.
- bromocresol green.
- bromocresol green solution--In a 100 mL volumetric flask dissolve 0.100 g bromocresol green in methanol. Dilute to volume with methanol.
- methyl red.
- methyl red solution--In a 100 mL volumetric flask dissolve 0.100 g methyl red in methanol. Dilute to volume with methanol.
- receiving solution (Tecator, 1985)--Dissolve 100 g H_3BO_3 in DI water and dilute to 10 L. Add 100 mL bromocresol green solution. Add 70 mL methyl red solution. Add 5 mL of 40% NaOH solution.
- recovery check solution, 5,000 mg-N/L--In a one litre volumetric flask dissolve 19.0927 g NH_4Cl in DI water. Dilute to volume.

Calibration and Standardization

Before analyzing the digested samples, distilled water blanks are run on the Kjeltec 1030 Analyzer until a constant reading of HCl is obtained. A 5 mL aliquot of a recovery check solution containing 5,000 mg-N/L is analyzed to check the recovery. Recovery should be within $\pm 10\%$.

Procedure

- Step 1 - Weigh 0.250 g of organic horizons, 0.500 g of Ah horizons (60 mesh), or 1 to 2.00 g of mineral soil (60 mesh) into a digestion tube.
- Step 2 - Add 10 mL concentrated H_2SO_4 to the tube and mix by swirling.
- Note:** This step should be carried out in a fume hood.
- Step 3 - Heat tubes at 200° C in the digestion block until very black (approx. 30 minutes).
- Step 4 - Add one catalyst tablet (Kjeltab).
- Step 5 - Heat tubes at 200° C for 15-20 minutes until the Kjeltab dissolves.
- Step 6 - Increase the block temperature to 300° C and heat for 30 minutes.
- Step 7 - Raise the temperature to 425° C and heat tubes until the sample turns a turquoise green. Digest samples for 20 minutes.
- Step 8 - Remove the digestion tubes from the block and allow to cool for about 5 minutes.
- Note:** Do not allow to cool in the heating block as NH_3 from the $(NH_4)_2SO_4$ formed by digestion will be lost if heated.

- Step 9 - Add approximately 30 mL DI water and mix well until sample is in solution.
- Step 10 - Dilute to approximately 100 mL with DI water.
- Step 11 - Follow instructions for the operation of the Kjeltec Auto 1030 Analyzer (Tecator, 1985).
- Step 12 - Set the alkali pump to deliver 25-30 mL of 40% NaOH.
- Step 13 - Titrate the sample with 0.01 M HCl.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger. It is recommended that two or more accuracy standards be prepared with each batch of samples. These provide a check on total between-run precision (digestion and distillation/titration).

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

Report total N as percentage on a dry-weight basis to the nearest 0.01% using the following formula:

$$N \% = \frac{(\text{mL sample} - \text{mL blank}) \times N \times 1.401}{\text{weight (g) of dry soil}}$$

where: N = normality of HCl titrant solution.

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Extractable Phosphorus

Introduction

Phosphorus (P) is an essential nutrient for plant growth and is required in relatively large quantities by plants. It is essential for energy transfer reactions within all cells and is a constituent of many proteins, nucleic acids, and coenzymes. Information on the phosphorus status of soils has long been regarded as being very important to the proper management of soils for agricultural purposes. More recently, it has become recognized as important in forest management as well. The availability of P to plants is influenced by soil pH. Phosphorus is most available to plants at a soil pH of 6 to 7. At pH lower than 6, P becomes fixed in iron and aluminum compounds, and, at pH above 7, calcium phosphates precipitate to an extent which limits P availability.

From the above discussion, it can be seen that soil acidification resulting from atmospheric deposition can influence the availability of P to plant species. Since P is an important macronutrient for plants upon which growth rates and general health depend, it should be monitored as a parameter for terrestrial monitoring programs.

Review of Methods

Extractants

Phosphorus is of concern largely in relation to its role in plant growth and, therefore, methods for extracting soil P should best reflect the plant's ability to extract P from the soil. Standardizing methods is difficult because the effectiveness of an extractant depends on soil type.

Many extractants have been used to extract plant available P. The most common, and perhaps the ones which best correlate with plant P uptake, are $\text{NaHCO}_3/\text{NaOH}$ (Olsen) and $\text{NH}_4\text{F}/\text{HCl}$ (Bray methods). The Olsen method is widely used on soils with high base status. The extractant decreases the amount of Ca in solution by causing precipitation of CaCO_3 , resulting in the dissolution of calcium phosphates. It also may remove some of the less strongly bound P from Al compounds. Since the method does not remove strongly bound P from Al and Fe oxides, the P levels obtained from acidic soils can be very low, often below detectable limits. The Bray method is more appropriate on acidic soils. The combination of HCl and NH_4F removes readily acid soluble P forms, including Ca-phosphates. The NH_4F dissolves Fe- and Al-phosphates which are prevalent in acid soils. In calcareous soils, CaCO_3 rapidly neutralizes the acid (Randall and Grava, 1971), and low estimates of available P result. Also, insoluble compounds may form as a result of reactions of CaF_2 with P (Smillie and Syers, 1972).

The objective of most extractions is to approximate the relative amount of P that may be available for plant growth, therefore, it is logical to use extractants which are similar to those within the rhizosphere, such as root exudates. Organic ions, such as oxalate, malate, citrate, and acetate, would be appropriate for such use. These extractants have not been extensively used for soil testing, but have been used in research and were found to be effective extractants (Lopez-Hernandez et al., 1986; Ball and Williams, 1968). Holford and Callis (1985b) found a combination of ammonium lactate-acetic acid to be a superior method for gauging plant response to soil P levels than other methods, including Bray 1, Bray 2, and NaHCO_3 , on moderately acid and alkaline soils in Australia. On acidic soils neutral fluoride extractants and 0.01 M sulphuric acid were better indicators of response than ammonium lactate-acetic acid.

Extraction Methods

When choosing an extraction method, it is necessary to consider the purpose of the measurements and the type of soils being monitored. Phosphorus availability to plants, particularly forest species, is of primary concern when studying the terrestrial effects of acidic atmospheric deposition. Detrimental effects would be seen first on soils which are both sensitive to acid deposition (low acid buffering, capacity) and which are already low in P. Moderately acidic and acidic brunisols and podzols meet these criteria and, therefore, the extractant chosen should be a good indicator of plant-available P for these soils. There must also be evidence available linking extractable P levels to growth of tree species. For these reasons, the Bray methods have been chosen as means of determining extractable P for the terrestrial effects related to LRTAP studies. Thomas and Peaslee (1973) describe $\text{NH}_4\text{F-HCl}$ (the Bray extractant) methods as being appropriate for soils with low to medium CEC that have been moderately to highly weathered, and Olsen and Sommers (1982) state that the Bray method is most successful on acid soils.

Numerous modifications to the originally described Bray method have been made to suit local conditions. These have resulted in considerable intralaboratory variation in shaking times, soil:extractant ratios, and extractant concentrations. In general, the amount of P extracted increases with increased shaking time and speed as well as with decreased soil:solution ratios.

The Bray 2 method increases the concentration of HCl from 0.025 N to 0.10 N in order to dissolve more Ca-phosphates. In acid and moderately acid soils, this does not appreciably improve the correlation to plant uptake, and, for these soils the Bray 1 method is appropriate and is widely used. Due to the common use of the Bray 1 method, and its appropriateness for acid and moderated acid soils it is the method suggested for use in terrestrial effects studies.

Olsen and Sommers (1982) suggest a 1:7 soil:solution ratio. This ratio would appear to be appropriate for acid and moderately acid soils, although lower ratios (i.e., 1:50) are more suited to less acidic soils. McKeague (1978) recommends a soil:solution ratio of 1:10 (2.5 g soil to 25 mL solution). We also recommend the 1:10 ratio as it will result in the extraction of slightly greater amounts of P, is more convenient, and will still correlate well with plant uptake of P.

Shaking times vary from 1 minute (Olsen and Sommers, 1982) to 30 minutes (Halford and Cullis, 1985a). Although more P is extracted with increased shaking times, Olsen and Sommers (1982) and McKeague (1978) use a standard one minute shaking time.

Analysis of Extract

Phosphorus in the extracting solution has most often been measured by colorimetric methods. Molybdate blue methods are the most sensitive and common. A phosphomolybdate complex is reduced to give a blue colour. The reducing agent chosen depends upon the solution P concentration, the concentration of interfering substances, such as arsenates and silicates, and the extracting solution. Olsen and Sommers (1982) and Sheldrick (1984) recommend the use of SnCl_2 as a reducing agent for analysis of Bray extracts. However, the coloured solution produced with SnCl_2 is only stable for about 2 hours and must be mixed immediately before each analysis. Furthermore, at a fixed concentration of the reducing agent, the absorbance degrades with increasing P concentration. Additionally, the solution can produce precipitates and coat the flow cell. The latter problem can be minimized by periodically cleaning the apparatus with acid (Smith and Scott, 1983).

Ascorbic acid as a reducing agent has for the most part replaced SnCl_2 in measurements and was recommended by the Canada Soil Survey Committee (McKeague, 1978). The reagent is stable for 24 hours and problems with precipitates are not encountered, however, it is not as sensitive as

SnCl_2 , because colour development is weaker. For soil monitoring programs, the sensitivity obtained with ascorbic acid is more than adequate. The ascorbic acid reduction method using ammonium molybdate to develop colour is, therefore, preferred for use in LRTAP monitoring programs.

Reference Method

The reference method for the determination of extractable P from soil samples for terrestrial effects of acid deposition is the use of 0.03 N NH_4F + 0.025 N HCl as an extract solution (Bray 1) followed by a colorimetric analysis using ammonium molybdate with ascorbic acid as a reducing agent. Automated segmented flow colourimetric systems are commonly used for the measurement of P and curve calibration programs used for determining the standard calibration curve. The procedure described here, however, describes a manual method, keeping equipment costs to a minimum.

Summary

A 2.5 g soil sample is shaken with 25 mL of extracting solution for 1 min. The solution is then filtered. Approximately 5 mL aliquot is transferred to a 25 mL volumetric flask and the pH is reduced to 5 using H_2SO_4 . Ammonium molybdate reagent is added and the content of P is determined colorimetrically. The percent transmittance of the solution is read using a colorimeter. The Bray 1 P is reported in mg/kg.

Interferences and Shortcomings

Some labs add carbon black to assure a clear filtrate with less interference for colorimetric determination of P. Olsen and Sommers (1982) do not suggest the use of carbon black and suggest extra filtration steps be used.

For calcareous soils, a 1:50 soil:extracting solution should be used. Incomplete dissolution of Ca-phosphates may still be observed such that a different method is required.

Boric acid is sometimes used to eliminate possible interference from fluorides. However, the routine use of boric acid in most soils has not been established.

Safety

All operations should be carried out in well-ventilated conditions. Protective clothing including eye protection should be worn at all times, and especially when handling concentrated acids. Special care must be taken when adding water to concentrated H_2SO_4 . Use sodium bicarbonate and water to neutralize and dilute spilled acids.

Apparatus and Equipment

- flasks, Erlenmeyer, 50 mL.
- dispenser, capable of accurately dispensing 25 mL.
- shaker, reciprocating, Eberbach or equivalent.
- filter paper, Whatman #42.

- colorimeter.
- colorimeter tubes, glass, 1 cm light path.
- balance, accurate to 0.001 g.

Reagents and Consumable Materials

- sulphuric acid (H_2SO_4), concentrated, reagent grade.
- hydrochloric acid (HCl), concentrated, reagent grade.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- hydrochloric acid (HCl), 0.5 N: Dilute 20.2 mL of concentrated HCl to a volume of 500 mL with DI water.
- ammonium fluoride (NH_4F), 1 N--In a one litre volumetric flask, dissolve 37 g of NH_4F in DI water. Dilute to volume with DI water. Store in a polyethylene bottle.
- extracting solution--Add 15 mL of 1.0 N NH_4F and 25 mL of 0.5 N HCl to 460 mL of DI water to give a solution of 0.03 N NH_4F and 0.025 N HCl.
- ammonium molybdate--Dissolve 20.0 g of $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 225 mL of concentrated H_2SO_4 . Dilute to 2 litres with DI water.
- ascorbic acid--Dissolve 1.76 g of ascorbic acid in 200 mL of DI water.

NOTE: This solution must be made fresh each day before use.

- P stock standard--In a 1 litre volumetric flask, dissolve 0.876 g of KH_2PO_4 (which has been dried for 24 hours at 80°C) in the extracting solution. Dilute to volume with extracting solution. This gives a 200 mg/L (w/v) P standard.

Calibration and Standardization

Calibration standards should be prepared in the extraction solution. Prepare a standard curve by plotting the transmittances of at least 5 standards (up to 10 standards are commonly used) against P concentration on semilogarithmic graph paper. Construct the calibration curve by finding the "best" fit line of the plotted standard concentrations. P is reported in mg/L.

Procedure

- Step 1 - Place 2.5 g of soil (air dried and sieved through 2 mm sieve) into a 50 mL Erlenmeyer flask.
- Step 2 - Add 25 mL of extracting solution. Shake for 1 minute
- Step 3 - Filter through Whatman #42 paper.

- Step 4 - Pipet an aliquot of the extract containing 1 to 20 μg of P (usually 5 mL) into a 25 mL volumetric flask.
- Step 5 - Add 0.5 mL of 5 N H_2SO_4 to reduce pH to 5.
- Step 6 - Add DI water to bring the volume to about 20 mL, then add 4 mL of ammonium molybdate reagent. Make to volume and mix.
- Step 7 - Read absorbance at 880 μm after 10 min. The colour is stable for 24 hours.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If

analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

To calculate the concentration of extractable P as follows:

$$\text{mg P/kg soil} = \text{mg P/mL solution} \times 10$$

This calculation assumes an aliquot of 5 mL of extracting solution was used.

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Cation Exchange Capacity

Introduction

Cation exchange capacity (CEC), usually expressed in milliequivalents (meq) per 100 g of soil, is a measure of the quantity of readily exchangeable cations neutralizing negative charge in the soil (Rhoades, 1982). Negative charge in the soil can be derived from several sources which fall into two categories: permanent and pH-dependent (or variable) charge. Permanent charge sites are the result of isomorphous substitution within the crystal structure of layer silicate minerals which are commonly referred to as clay minerals. Permanent charge CEC is independent of pH, electrolyte concentrations, ion valences and the dielectric constant of the medium. The pH dependent CEC is derived from broken bonds at mineral edges, dissociation of acidic functional groups in organic matter and sesquioxides present in the soil, and preferential adsorption of certain ions on the charged particle surfaces. In general, as the pH, electrolyte concentration, dielectric constant of the medium, and the ion valences increase, the net contribution of pH-dependent charge to the overall CEC increases.

A close approximation of the CEC can be made by the summation of the exchangeable base cations of Ca^{2+} , Mg^{2+} , Na^+ , and K^+ and the exchangeable acidity as determined by BaCl_2 -TEA extraction or the addition of exchangeable Al extracted by KCl (USDA/SCS, 1972).

Review of Methods

Most CEC methods begin with the displacement of existing cations with a saturating salt to provide one index cation on the exchange complexes. Many different replacing cations have been used to study the exchange characteristics of soils. Some of these include NH_4^+ (Grove et al., 1982; Mroz et al., 1985; Richter, 1986; Johnson et al., 1991; Soil Survey Laboratory Staff, 1992), K^+ (Gillman and Uehara, 1980), Ba^{2+} (Kalisz and Stone, 1980; Rhoades, 1982; Hendershot and Duquette, 1986; Ross and Bartlett, 1992), and Sr^{2+} (Matsue and Wada, 1985). After removing or accounting for excess saturating salt, the index cation is replaced with a different saturating salt and subsequently measured to provide an estimate of the CEC of soil.

Although the choice of the replacing cation is considered arbitrary (Thomas, 1982), the selection of accompanying anions can be important. If the final replacing solution contains SO_4^{2-} , specific adsorption of this anion can affect the measured CEC in SO_4^{2-} adsorbing soils (Matsue and Wada, 1985; Hendershot and Duquette, 1986). Although some concern has been expressed regarding the selection of Cl^- as the replacement anion (due to ion pairing with divalent cations), Rhue and Reve (1990) did not see any differences in measured CEC when compared to ClO_4^- . Large differences have been found between soil CEC measured in buffered (pH 7) acetate salts when compared to neutral salts (Kalisz and Stone, 1980; Grove et al., 1982). In acid forest soils, the recommendation has been made to measure CEC with unbuffered salts (Rhoades, 1982; Hendershot et al., 1993). The concentration of the unbuffered salts has also been shown to influence the measured CEC (Wada and Okamura, 1980).

Two saturating solutions containing NH_4^+ are commonly used for CEC determination. Ammonium acetate (1.0 N NH_4OAc) buffered at pH 7.0 yields a CEC which is close to the total cation exchange capacity for a specific soil. This saturating solution is commonly used for soil comparisons because it has the advantage of extracting all samples at the same pH (USDA-SCS, 1972). In acid soils, this estimate results in a high CEC value relative to the CEC found under field conditions because of adsorption of NH_4^{++} ions to the pH-dependent exchange sites that exist above the soil's natural pH level (Grove et al., 1982). The overestimation will not occur when an unbuffered neutral salt solution such as ammonium chloride (1.0 N NH_4Cl) is used. During the extraction, the

solution assumes the pH of the soil. The NH_4Cl CEC has been termed "effective" CEC because it provides more unambiguous information about the behaviour of the soil under field conditions.

Reference Method

The reference approach to long-term monitoring methods for cation exchange capacity is to measure the effective CEC with 1 N NH_4Cl . Buffered salt solutions will not be used as they are not considered representative of the field forest soil condition. A 1 N NH_4Cl salt solution was chosen over other commonly used solutions (NaCl , KCl , and BaCl_2) due to its predominant use for extracting exchangeable cations in studies of forested ecosystems in North America.

Summary of Method

The soil sample is saturated with NH_4^+ from a solution of NH_4Cl . Excess NH_4^+ is removed by ethanol rinses. The NH_4^+ is displaced by Na^+ and is measured by one of three methods: automated distillation-titration, manual distillation-automated titration, or ammonium displacement-flow injection analysis. These methods are based on Doxsee (1985), Rhoades (1982), and USDA/SCS (1984).

The NH_4Cl saturating solution should be retained for the exchangeable cation determinations (Chapter 13).

NOTE: This method has been written assuming the use of a mechanical extractor.

Interferences and Shortcomings

Inconsistency in the NH_4^+ saturating and rinsing steps is the greatest source of error. Soils containing an abundance of minerals, such as biotite, muscovite, illite, and vermiculite, may retain interlattice NH_4^+ and produce artificially high results. The use of a mechanical extractor minimizes inconsistency.

Safety

Wear protective clothing (laboratory coat and gloves) and safety glasses when preparing reagents, especially when concentrated acids and bases are used. The use of concentrated acids and hydroxide solutions should be restricted to a fume hood.

Apparatus and Equipment

Apparatus for Saturation Procedure

- mechanical extractor, 24-place (Figure 1).
- reciprocating shaker.
- balance, capable of weighing to 0.01 g.
- balance calibration weights, 3-5 weights covering expected range.
- wash bottle.

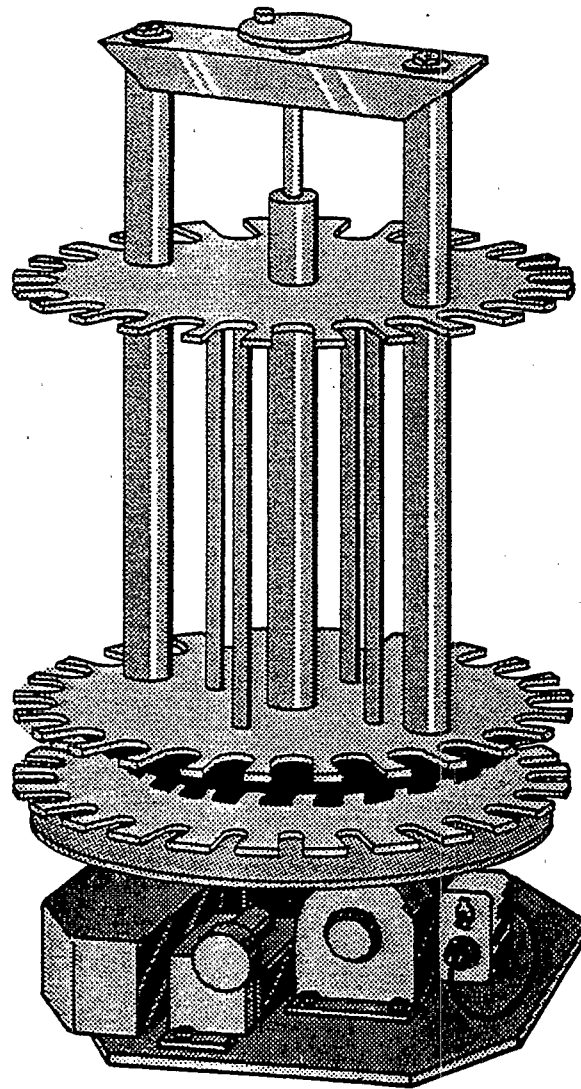


Figure 1. Mechanical Extractor.

Apparatus for Analysis

The apparatus and equipment needed are specific to the selected analytical method. It is not necessary to have equipment for all three analytical methods.

Ammonium Displacement-Flow Injection

- flow injection analyzer (FIA), Lachat or equivalent, modified for ammonium chemistry with 630 nm interference filter and consisting of:
 - a. sampler.
 - b. analytical manifold with 200 μ L sample loop.
 - c. in-line heater.
 - d. colorimeter equipped with a 10 mm flow cell.
 - e. printer.
- balance, capable of weighing to 0.001 g.
- balance calibration weights, 3-5 weights covering expected range.

Automated Distillation-Titration

- steam distillation-titration apparatus, Kjeltac Auto 1030 Analyzer, or equivalent.
- printer, Alphacom 40, or equivalent.
- digestion tubes, 250 mL, straight neck.
- balance, capable of weighing to 0.1 g.
- balance calibration weights, 3-5 weights covering expected range.
- policeman, rubber.

Manual Distillation/Automated Titration

- automatic titrator with autosampler, Metrohm or equivalent.
- Kjeldahl flasks, 800 mL.
- balance, capable of weighing to 0.1 g.
- balance calibration weights, 3-5 weights covering expected range.
- policeman, rubber.

Reagents and Consumable Materials for Saturation Procedure

- washed analytical filter pulp, Schleicher and Schuell, No. 289 (see procedure section for washing procedure).
- glacial acetic acid ($\text{HC}_2\text{H}_3\text{O}_2$), concentrated, reagent grade.
- ammonium hydroxide (NH_4OH), concentrated, reagent grade.
- ammonium chloride (NH_4Cl), reagent grade, 1 N -- Dissolve 535 g in DI water and dilute to 10 L.
- ethanol ($\text{C}_2\text{H}_5\text{OH}$), 95 percent, U.S.P.
- Nessler's reagent--

Add 4.56 g potassium iodide (KI) to 30 mL DI water in a beaker. Add 5.68 g mercuric iodide (HgI_2). Stir until dissolved.

Dissolve 10 g NaOH in 200 mL DI water.

Transfer NaOH solution to 250 mL volumetric flask. Add Hg solution slowly. Dilute to volume and mix thoroughly. Solution should not contain a precipitate. It can be used immediately.

- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- syringes, disposable, 60 mL polypropylene (use one sample tube, one reservoir tube, and one tared extraction syringe for each sample).
- rubber tubing, 1/8 x 1/4 inch (for connecting syringe barrels).
- bottles, linear polyethylene (LPE), 25 mL.
- tubes, glass, centrifuge or culture, with caps, 25 mL.
- weighing pans, disposable.

Reagents and Consumable Materials for Analysis

The reagents and consumable materials needed are specific to the selected analytical procedure. It is not necessary to have the reagents and materials for all three analytical procedures.

Ammonium Displacement-Flow Injection Analysis

The reagents and consumable materials used depend on recommendations of the manufacturer of the FIA and may vary by make and model.

- hydrochloric acid, 0.1 N--Purchased or prepared by the following procedure: Add 150 mL concentrated HCl to approximately 15 L DI water, dilute to 18 L. Standardize 0.1 N HCl by titration against dried primary standard grade sodium carbonate to the methyl orange end-point.
- nitroferricyanide reagent--Dissolve 40 g potassium sodium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6$) and 30 g sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) in 500 mL DI water. Add 10 g sodium hydroxide pellets (NaOH). Add 1.5 g sodium nitroferricyanide ($\text{Na}_2\text{Fe}(\text{CN})_6\text{NO} \cdot 2\text{H}_2\text{O}$), dilute to 1.00 L, and mix well. Store in a dark bottle. Prepare fresh solution monthly.
- sodium hypochlorite reagent--Dissolve 20 g sodium hydroxide and 20 g boric acid in 150 mL of DI water. Add 800 mL 5 percent solution NaOCl. Dilute to 1.00 L with DI water. Store in a dark bottle. Prepare fresh solution monthly.
- sodium phenate reagent--Dissolve 95 mL of 88 percent liquified phenol in 600 mL DI water. While stirring, slowly add 120 g NaOH. Cool. Add 100 mL ethanol and dilute to 1.00 L. Store in a dark bottle.
- nitrogen standard solution, 1,000 mg $\text{NH}_4\text{-N/L}$ --Dissolve 3.819 g ammonium chloride (NH_4Cl), dried at 105° C, in DI water and dilute to 1.00 L.
- working standards--Pipet 15.0, 10.0, 6.0, and 2.0 mL of the nitrogen standard solution, above, into 100 mL volumetric flasks. Bring to volume with 0.1 N HCl. This will yield 150, 100, 60, and 20 mg $\text{NH}_4\text{-N/L}$ working standards. Pipet 5 mL of the 100 mg $\text{NH}_4\text{-N/L}$ working standard into a 100-mL volumetric flask and dilute to volume with 0.1 N HCl. This provides a 5 mg $\text{NH}_4\text{-N/L}$ working standard. Prepare fresh working standards weekly.
- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- weighing pans, disposable.

Automated Distillation-Titration

- sodium chloride (NaCl).
- antifoam, silicone spray bottle.
- hydrochloric acid (HCl), 0.10 N, standardized.
- boric acid (H_3BO_3), 4 percent (w/v) aqueous solution--Add 720 g boric acid to about 4 L DI water in a large stainless steel beaker. Heat to near boiling and stir until crystals dissolve. Add to a 5 gallon Pyrex bottle about 12 L DI water. Transfer hot solution through a large polyethylene funnel into the bottle. Dilute to 18 L with DI water and mix well.
- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- compressed air.
- weighing pans, disposable.

Manual Distillation-Automatic Titration

- sodium chloride (NaCl).
- antifoam mixture--Mix equal parts of mineral oil and octanol.
- boric acid (H_3BO_3), 4 percent.
- hydrochloric acid (HCl), 0.1 N.
- sodium hydroxide (NaOH), 1 N--Add 500 mL 50 percent NaOH solution to 8 L of DI water in a 9.5 L Pyrex solution bottle. Dilute to 9 L with DI water and mix well.
- zinc, granular.
- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- compressed air.
- beakers, plastic disposable, 250 mL.
- weighing pans, disposable.

Calibration and Standardization

Calibration standards should be prepared in the extraction solution.

Flow Injection Analysis Calibration

For the FIA, use standards containing 0, 5, 20, 60, 100, and 150 mg $\text{NH}_4\text{-N/L}$ to develop a calibration curve. A regression of the standard curve should have an intercept close to zero. Air bubbles can produce sharp sudden peaks which destroy the calibration curve. In the event of air bubbles, the calibration curve and all samples analyzed since the last quality control check sample (QCCS) must be reanalyzed. Standard values should not vary by more than 5 percent relative standard deviation (%RSD).

Titration Calibration

Titants used in the automated titrations are calibrated prior to analysis to establish the normality. The normality is checked weekly. Should the check value differ from the normality by more than 5 percent, two additional checks are run and the mean of the three check values is used as the normality. The same standard titrant should be used for all samples within a batch.

Procedure

Before proceeding with the analytical procedure, the analyst should be certain that all QC procedures have been implemented, all labware has been cleaned properly, and valid instrumental detection limits (IDLs) have been obtained.

NOTE: This method has been written assuming the use of a mechanical extractor.

Pulp Washing

NOTE: Commercial filter pulps often are contaminated and will have to be washed.

- Step 1 - Prepare sample tubes by tightly compressing a 0.5 g ball of filter pulp into bottom of syringe barrel with a modified plunger. Modify the plunger by removing the rubber portion of the plunger, and cut off the plastic protrusion.
- Step 2 - Add 50 mL 0.1 N HCl to the syringe containing the pulp and extract rapidly.
- Step 3 - Add 50 mL DI water to the syringe containing the pulp and extract rapidly.
- Step 4 - Repeat Step 3 again.
- Step 5 - Remove any excess solution from the washed pulp by applying gentle suction to the syringe tip. Proceed immediately with an extraction.

Extraction

- Step 1 - *For mineral soils*, weigh 5.00 g air-dry soil, place in sample tube, and record exact weight. Place sample tube in upper disc of extractor and connect to inverted, tared extraction syringe, the plunger of which is inserted in the slot of the stationary disc of the extractor. Fill the syringe to the 25 mL mark with NH_4Cl . Stir sample and NH_4Cl with glass stirring rod for 15 seconds, rinse rod with NH_4Cl , and fill syringe to the 30 mL mark. Let stand for 20 minutes.

For organic soils, weigh 1.25 g of air-dried soil into a small glass tube and record exact weight. Add 2 mL ethanol as a wetting agent. (If the organic soil wets easily, it is not necessary to add the ethanol.) When the soil is moistened, add 15 mL NH_4Cl , cap, and shake for one hour on a reciprocating shaker. Place sample tube in upper disc of extractor and connect to inverted, tared extraction syringe, the plunger of which is inserted in the slot of the stationary disc of the extractor. Then quantitatively transfer the sample and NH_4Cl to the sample tube and fill to the 25 mL mark with NH_4Cl . Let stand for 20 minutes.

NOTE: Up to 35 mL may be used for transfer; see Step 2.

- Step 2 - Put reservoir tube on top of sample tube; extract rapidly until NH_4Cl is at a depth of 0.5 to 1.0 cm above sample. Turn off extractor. Add about 45 mL NH_4Cl to reservoir tube, turn on extractor, and extract overnight or for approximately 16 hours. Do not allow the soil to dry between the time the extractor is turned off and back on.

NOTE: If 35 mL are used in Step 1, reduce 45 mL to 35 mL. Total NH_4Cl used during extraction should be approximately 70 mL.

- Step 3 - The next morning, switch off extractor and pull plungers down as far as extractor will allow. Disconnect syringes from sample tubes, leaving rubber connectors on sample tubes. Weigh each syringe containing the NH_4Cl extract to the nearest 0.01 g. The final weight and tare weight are used to calculate the volume of ammonium acetate extract (Section 12.0), according to the formula in the calculation and reporting section.

- Step 4 - Mix the extract in each syringe by shaking manually. Rinse the appropriately labeled polyethylene bottle twice with small volumes of the extract solution, then fill the bottle with extract solution and discard the excess. This solution is reserved for analysis of exchangeable cations as described in Chapter 13.
- Step 5 - Return upper 2-disc unit to starting position. Attach the syringes to the sample tubes, and rinse the sides of sample tubes with ethanol from a wash bottle. Fill sample tubes to the 20 mL mark, stir, and let stand for 15 to 20 minutes. Place reservoir tube in sample tube. Extract rapidly until the level of ethanol is 0.5 to 1.0 cm above sample. Turn off extractor and add enough ethanol to the reservoir to ensure an excess over the capacity of the syringe. Extract for 45 minutes.
- Step 6 - After the extractor has stopped, turn off the switch, pull the plungers down, remove syringes, and discard the ethanol wash. Return the upper unit of the extractor to starting position, reattach syringes to the sample tube, fill reservoir tubes with about 45 mL ethanol, and extract a second time for approximately 45 minutes. When extractor has stopped, remove syringes and discard ethanol. After the second ethanol extraction, collect a few drops of ethanol extract on a spot plate. Test for residual NH_4^+ in each sample by adding four drops of Nessler's reagent to one drop of solution. If the test is positive (i.e., orange endpoint) repeat another ethanol extraction of the affected samples and test by using Nessler's reagent until a negative test is obtained.

Analytical Procedure Using FIA

- Step 1 - Add 50 mL of 0.1 N HCl and extract at a setting of 10 (approximately one hour) and record volume.
- Step 2 - Disconnect syringes and save the HCl extract for FIA analysis.
- Step 3 - Operate the FIA according to manufacturer's instructions.
- Step 4 - Read mg $\text{NH}_4\text{-N/L}$; if concentrations exceed calibration standards, dilute in the instrument calibration range and reanalyze.

Analytical Procedure using Automated Distillation-Titration

- Step 1 - Remove sample tubes and quantitatively transfer each sample to a 250 mL digestion tube. To remove the sample, blow the filter pulp and soil out of the syringe by using a gentle flow of compressed air. Wash with a minimum of DI water. Use a rubber policeman to complete the transfer.
- Step 2 - Add 6 to 7 g sodium chloride to the digestion tube, spray silicone antifoam solution into the digestion tube and connect it to the analyzer.
- Step 3 - Follow instruction manual regarding safety and operation of the analyzer and titrate with 0.1 N HCl to a pH 4.60 endpoint.
- Step 4 - Read mL titration and record with the normality of titrant.

Analytical Procedure using Manual Distillation-Automated Titration

- Step 1 - Transfer sample to an 800 mL Kjeldahl flask.
- Step 2 - Add 400 mL DI water, 10 g NaCl, 5 drops antifoam mixture, 1 to 2 g granular zinc, and 40 mL 1.0 N NaOH.
- Step 3 - Set up Kjeldahl distillation apparatus and distill until 175-180 mL of distillate is collected in a 250 mL plastic beaker containing 50 mL of 4 percent boric acid solution.
- Step 4 - Transfer plastic beaker to distillation apparatus and operate according to manufacturer's instructions.
- Step 5 - Read mL titration and record with the normality of titrant.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. This standard should have concentrations at about mid-calibration range. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

Flow Injection Analysis

$$\text{CEC (meq/100 g)} = \{([(\text{Final Sol. Vol.}] \times [\text{Inst. Reading}]) \times ([\text{Total Diluted Vol.}] + [\text{Aliquot Vol.}] \times 1 \text{ L}) + \{[1,000 \text{ mL} \times 1 \text{ meq} + 14 \text{ mg} \times 1] + (\text{sample wt.} \times (1 - [\text{MOIST}])) + (100 + [\text{MOIST}])\}$$

Titration

$$[\text{CEC (meq/100 g)}] = \{([\text{Titrant Volume}] \times [\text{Normality}] \times 1) + \{[\text{sample wt.}] \times (1 - [\text{MOIST}])\} \div (100 + [\text{MOIST}]) \times 100\}$$

NOTE: moisture corrections [MOIST] are applicable if sample extracted is not oven-dried, thereby, removing all free water. The moisture correction factor is as follows:

$$[(1 - \text{moisture content in \%}) \div (100 + \text{moisture content in \%})]$$

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Exchangeable Cations

Introduction

Exchangeable cations are those cations (positively charged ions) in soil that can be exchanged with cations of an added salt solution. They are comprised of exchangeable bases and exchangeable acidity. The quantity of the exchangeable cations required to neutralize the negative charge in soil is defined as the cation exchange capacity (CEC). The negative charge in soil arises from factors such as isomorphous substitution within layer silicate minerals, broken chemical bonds on the edges and external surfaces of soil minerals, and the dissociation of acidic functional groups in soil organic compounds.

Exchangeable bases commonly found in soils, presented in order of relative abundance, include calcium, magnesium, potassium, and sodium. Exchangeable acidity is primarily composed of exchangeable aluminum and hydrogen. The exchange complex of most soils falls somewhere between being saturated with basic or acidic cations. The fraction of the CEC that is comprised of exchangeable bases is defined as the base saturation and is extremely important in determining whether a soil is relatively acidic or basic. Soil acidification occurs when base cations are replaced with acidic cations in the soil.

The exchangeable cations are essential nutrients for plant growth. Acidic inputs to soil result in a replacement of the base cations with acidic cations. A concern for forest health is that acidic deposition could result in a diminishing supply of essential nutrients and a simultaneous increase in aluminum due to increased Al solubility that can be toxic to plant roots. Andersson (1986) concluded that acidic deposition has contributed to an accelerated decline in the base saturation of several soils in Sweden over the period of 1949-1984. Nutrient deficiencies of magnesium and calcium have been correlated to key symptoms of forest decline in Europe (Krause et al., 1986).

As exchangeable cations are leached from the soil, the soil loses its ability to buffer further acidic inputs. In the absence of other buffering mechanisms such as sulphate adsorption, soil water tends to become more acidic and increase in its concentration of aluminum and heavy metals. The decline in base saturation in soils therefore has important implications to the quality of streams and lakes in forested ecosystems (Reuss and Johnson, 1986).

Review of Methods

By definition, any added cation should be able to replace an exchangeable cation in the soil. It is for this reason that the choice of the replacement cation has often been considered as somewhat arbitrary. The consequence is that a tremendous variety of replacement solutions have been used by soil scientists for the characterization of exchangeable cations in soil. The only cations that might be excluded from the selection process are those one would wish to measure such as Ca^{2+} , Mg^{2+} , K^+ , Na^+ , and Al^{3+} . The only exception to this approach is that Na^+ is used in certain soil regions where its contribution to the exchangeable bases is minimal. The most common replacement cations are NH_4^+ , Ba^{2+} , and Sr^{2+} although Li^+ , K^+ , and Cs^+ have all been used.

The anion in the replacement solution cannot be considered as an arbitrary choice. Anions can be selected to provide a neutral salt solution such as with Cl^- or SO_4^{2-} salts or a pH buffered salt solution such as 1 N NH_4OAc (pH 7). The quantity of acidic cations, such as aluminum, in soils is very dependent on the pH of the soil. Any anion that alters the soil pH will alter the amount of exchangeable acidity measured as well as the CEC.

The concentration of the replacement solution can have an impact on measured soil properties such as the CEC. Many factors affect the measured CEC of a soil including the pH of the extracting solution, the electrolyte level, the valence (charge) of the counter ion, and the dielectric constant of the extracting and wash solutions. The magnitude of a change in measured CEC for any of these factors depends on the soil organic matter content and the predominant clay minerals present in the soil.

The measurement of exchangeable acidity and CEC can be approached in two different ways. One approach is to try and estimate the "effective" acidity or CEC or that measurement which simulates as close as possible the actual condition in the field. This is generally accomplished with neutral unbuffered salts. A recent technique has been to use an unbuffered BaCl_2 salt to saturate the soil and then measure the CEC of the soil at soil solution concentration similar to a field soil through compulsive replacement of Ba with the addition of a dilute solution of MgSO_4 (Rhoades, 1982). An alternative approach is to measure the "total potential" acidity or CEC by buffering the soil to pH 8.3.

A wide variety of soil:solution ratios have been used in the measurement of exchangeable cations. The main constraint is that an excess of cations be added to effectively replace the exchangeable cations in the soil. The equilibration time with the soil also varies considerably from a few minutes to overnight extractions. Filtering or centrifuging are both used to obtain a clean extract for chemical analysis after equilibration with the soil. Filtering tends to provide a cleaner solution as organic materials may float in solutions which are centrifuged. A disadvantage of filter materials is that they may be found to contaminate the extract with low levels of base cations and may have a measurable CEC. For this reason, it is important to run reagent blanks (extractions without soil) to test for possible contamination in the extraction process.

The majority of CEC methods require a displacement of the saturating ion by another salt solution. The displacement is often accomplished after the soil has been washed free of excess saturating solution. The solutions used to wash the soil vary from deionized water to alcohol solutions (methyl, ethyl, or isopropyl alcohols).

Reference Method

The reference approach to long-term monitoring methods for exchangeable bases is to measure the exchangeable bases, effective acidity, and effective CEC with 1 N NH_4Cl . Buffered salt solutions will not be used as they are not considered representative of the forest soil condition. A 1 N NH_4Cl salt solution was chosen due to its predominant use for extracting exchangeable cations in studies of forested ecosystems in North America. The NH_4^+ ion will not interfere with the measurement of any of the exchangeable cations and has been shown (Robarge, 1988) to be an essentially equivalent extractant of exchangeable Al as 1 N KCl (the most common exchangeable Al extractant). The use of just one extracting solution also reduces the time and analytical requirements for measurements. A compulsive replacement method is not being recommended as it is felt that it would be too time consuming and operator dependent for long-term monitoring purposes.

The effective CEC is measured by replacing the NH_4^+ ion with Na^+ after washing the soil with 50% ethyl alcohol to remove excess soil solution NH_4^+ ion and testing for an absence of Cl in the wash with a AgNO_3 .

Two choices for extraction procedures are provided in the methods section. In addition to a common equilibration procedure that does not require any specialized equipment, an automated

equilibration procedure is also included as many of the soil laboratories in Canada are presently using this equipment.

Summary of Method

Previously prepared extracts from the CEC procedure are analyzed for aluminum, calcium, magnesium, potassium, and sodium. Once the concentration of each cation in the soil extract is determined, the cation concentrations in the original soil sample may be calculated.

Atomic absorption spectroscopy (AAS) can be used to measure calcium, magnesium, potassium, and sodium. A light beam from a hollow cathode lamp, whose cathode is made of the element to be determined, is directed through the flame into a monochromator and onto a detector that measures the amount of light passing through the flame. Absorption depends upon the presence of free, unexcited, ground state atoms in the flame. Since the wavelength of the light beam is characteristic only of the cation being determined, the light energy absorbed by the flame is a measure of the concentration of that cation in the extract.

Inductively coupled plasma spectroscopy (ICP) must be used to measure aluminum and can be used to measure calcium, magnesium, and sodium. Samples are nebulized to produce an aerosol. The aerosol is transported by an argon carrier stream to an inductively coupled argon plasma, which is produced by a radio frequency generator. In the plasma (which is at a temperature of 6,000 to 10,000° K), the analytes in the aerosol are atomized, ionized, and ionic emission spectra is produced. The spectra from all analytes are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed by a computer system. The signal is proportional to the analyte concentration and is calibrated by analyzing a series of standards (U.S. EPA, 1983; Fassel, 1982).

Emission spectroscopy (ES) can be used to measure potassium and sodium. The sample is aspirated into a gas flame and excitation is carried out under carefully controlled and reproducible conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices such as prisms or gratings. The intensity of light is measured by a phototube potentiometer or other appropriate circuit. The intensity of light at the appropriate wavelength (e.g., 589 nm for Na⁺) is approximately proportional to the concentration of the element.

Interferences and Shortcomings

No interferences are known to occur during extraction process, but several interferences may occur during analysis of the exchangeable cations, including spectral, chemical, physical, and matrix effects.

Spectral interferences are generally caused by spectral overlap from another element or background contributions. These interferences can usually be corrected by monitoring and compensating for the effect of interfering elements, selecting another wavelength, correcting background effects, or using a narrower slit width.

Chemical interferences are often caused by the cations forming molecular compounds instead of dissociated ions. This interference, most pronounced with the multivalent ions (such as Ca²⁺, Mg²⁺, and especially Al³⁺), is negligible with the ICP technique. This interference is often corrected in AAS by the addition of lanthanum or lithium or by the avoidance of anions such as sulphate and phosphate in the extractant.

The most common physical interference in the analysis of soils exchange solutions is salt build-up clogging the burner or nebulizer. Although dilution will reduce this problem, it will also change the matrix and any effect it may have on the instrument read-out.

Matrix effects are usually compensated for by analyzing samples and all calibration standards, method blanks, and quality control standards in the same matrix. Matrix effects may be tested by serial dilutions, spiked additions, and comparison with an alternative method of analysis. When the matrix effects are significant and cannot be readily corrected, the analyses must be performed by standard additions.

Safety

Wear protective clothing (laboratory coat and gloves) and safety glasses when preparing reagents, especially when concentrated acids and bases are used. The use of concentrated acids and hydroxide solutions should be restricted to a fume hood. Many metal salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling.

Follow the safety precautions of the manufacturer when operating instruments. Gas cylinders should always be chained or bolted in an upright position.

Apparatus and Equipment

Determination by Atomic Absorption

- spectrophotometer, with grating monochromator, photomultiplier detector, adjustable slits, and a wavelength range of 190 to 800 nm.
- burner, as recommended by the spectrophotometer manufacturer. When nitrous oxide is used as the oxidant, a nitrous oxide burner is required.
- hollow cathode lamps, single element lamps preferred; multi-element lamps may be used. Electrodeless discharge lamps may be used where available.
- balance, capable of weighing to 0.1 g.
- balance calibration weights, 3-5 weights covering expected range.

Determination by Inductively Coupled Plasma

- inductively coupled plasma-atomic emission spectrometer.
- balance, capable of weighing to 0.01 g.
- balance calibration weights, 3-5 weights covering expected range.

Determination by Emission Spectroscopy

- flame photometer, direct-reading or internal-standard type; or an atomic absorption spectrometer operated in the flame emission mode.

- balance, capable of weighing to 0.001 g.
- balance calibration weights, 3-5 weights covering expected range.

Reagents and Consumable Materials

Acids used in the preparation of standards and for sample processing must be of ultra-high purity grade (e.g., Baker Ultrex grade or SeaStar Ultrapure grade). To minimize concentration of cations in standard solutions by evaporation, store solutions in linear or high density polyethylene bottles. Use small containers to reduce the amount of dry element that may be picked up from the bottle walls when the solution is poured. Shake each container thoroughly before use to redissolve any accumulated salts from the walls.

Deionized (DI) water used for preparing or diluting reagents, standards, and samples must meet purity specifications for Type I reagent water as given in ASTM D 1193 (ASTM, 1984).

Determination by Atomic Absorption

- hydrochloric acid, concentrated (12 M HCl)--Ultrapure grade, Baker Instra-Analyzed or equivalent.
- HCl (1 percent v/v)--Add 5 mL concentrated HCl to 495 mL DI water.
- nitric acid, concentrated--Ultrapure grade, Baker Instra-Analyzed or equivalent.
- HNO₃ (0.5 percent v/v HNO₃)--Add 0.50 mL HNO₃ to 50 mL DI water and dilute to 100 mL.
- primary standard solutions--Prepare from ultra-high purity grade chemicals as directed in the individual procedures. Commercially available stock standard solutions may also be used.
- dilute calibration standards--Prepare a series of standards of the cation by dilution of the appropriate stock metal solution in the specific matrix to cover the concentration range desired. Prepare all calibration standards in concentration units of mg/L.
- fuel--Commercial grade acetylene with in-line filter is generally acceptable.
- oxidant--Air may be supplied from a compressed-air line, a laboratory compressor, or from a cylinder of compressed air. Nitrous oxide is supplied from a cylinder of compressed gas.
- lanthanum chloride (LaCl₃) matrix modifier solution--Dissolve 29 g La₂O₃, slowly and in small portions, in 250 mL of concentrated HCl.

Caution: Reaction is violent. Dilute to 500 mL with DI water.

- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- weighing pans, disposable.

Determination by Inductively Coupled Plasma

- hydrochloric acid, concentrated (12 M HCl, specific gravity 1.19)--Ultrapure grade, Baker Instra-Analyzed or equivalent.
- HCl (50 percent v/v)--Add 500 mL concentrated HCl to 400 mL DI water and dilute to 1.00 L with DI water.
- nitric acid, concentrated (specific gravity 1.41)--Ultrapure grade, Baker Instra-Analyzed or equivalent.
- HNO₃ (50 percent v/v)--Add 500 mL concentrated HNO₃ to 400 mL DI water and dilute to 1 L.
- primary standard solutions--May be purchased or prepared from ultra-high purity grade chemicals or metals. All salts must be dried for one hour at 105° C unless otherwise specified.
- argon, oxygen-free.
- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- weighing pans, disposable.

Determination by Emission Spectroscopy

- standard lithium solution--Use either lithium chloride (LiCl) or lithium nitrate (LiNO₃) to prepare standard lithium solution containing 1,000 mg Li/L.

NOTE: Lithium is used to suppress ionization of K⁺ and Na⁺.

Dry LiCl overnight in an oven at 105° C. Rapidly weigh 6.109 g LiCl and dissolve in 1.0 N NH₄Cl, as needed to match the sample extract matrix. Dilute to 1,000 mL with the same 1.0 N NH₄Cl solution.

Dry LiNO₃ overnight in an oven at 105° C. Rapidly weigh 9.935 g LiNO₃ and dissolve in 1.0 N NH₄Cl, as needed to match the sample extract matrix. Dilute to 1,000 mL with the same 1.0 N NH₄Cl solution.

Prepare a new calibration curve whenever the standard lithium solution is changed. Do not change solutions within a batch.

- primary standard solutions--May be purchased or prepared from ultra-high purity grade chemicals or metals. All salts must be dried for one hour at 105° C unless otherwise specified.
- acetylene (commercial grade or better).

- water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- weighing pans, disposable.

Calibration and Standardization

Calibration standards should be prepared in the extraction solution for the various cations. Within each class of instruments (AAS, ICP, and ES), the calibration procedure varies slightly. Calibrate by analyzing a calibration blank (0 mg/L standard) and a series of at least three additional standards within the linear range of the instrument. If an ICP is used, a multi-element standard may be prepared and analyzed. For AA and ES determinations, the instrument must be calibrated for each analyte by using a separate standard. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded.

Procedure

Before proceeding with the analytical procedure, the analyst should be certain that all QC procedures have been implemented, all labware has been cleaned properly, and valid instrumental detection limits (IDLs) have been obtained.

General procedures for AA, ICP, and ES are given in following sections.

Procedure for Determinations by Atomic Absorption Spectrometry

Differences among AA spectrophotometers prevent the formulation of detailed instructions applicable to every instrument. The analyst should follow the operating instructions for his particular instrument. In general, after choosing the proper hollow cathode lamp for the analysis, allow the lamp to warm up for a minimum of 15 minutes unless the instrument is operated in a double-beam mode. During this period, align the instrument, position the monochromator at the correct wavelength, select the proper monochromator slit width, and adjust the hollow cathode current according to the recommendation of the manufacturer. Subsequently, light the flame and regulate the flow of fuel and oxidant, adjust the burner and nebulizer flow rate for maximum percent absorption and stability, and balance the photometer. Run a series of standards of the analyte and calibrate the instrument. Aspirate the samples and determine the concentrations either directly (if the instrument reads directly in concentration units) or from a calibration curve.

Calcium and Magnesium

Step 1 - Add 1.0 mL of LaCl_3 solution to a 10-mL volume of each calibration standard, method blank, QC sample, and routine sample. Dilute with DI water to 20 mL.

Step 2 - Calibrate the instrument.

Step 3 - Analyze the samples.

NOTE: Initial readout should be made on the 1:1 soil extract:DI water dilution *before* any additional dilutions are made. For further dilutions, use a 1:1 DI water:extraction solution (i.e., 50 percent extracting solution) as the dilution agent.

- Step 4 - Dilute and reanalyze any samples for which the concentration exceeds the linear range, as outlined in Step 1.
- Step 5 - Record results as mg/L in the soil extract.

Potassium and Sodium

- Step 1 - Calibrate the instrument.
- Step 2 - Analyze the samples.

NOTE: Initial readout should be made on the 1:1 soil extract:DI water dilution *before* any additional dilutions are made. For further dilutions, use a 1:1 DI water:extraction solution (i.e., 50 percent extracting solution) as the dilution agent.

- Step 3 - Dilute and reanalyze any sample for which the concentration exceeds the calibrated range, and record aliquot and diluted volumes.
- Step 4 - Record results as mg/L in the soil extract.

Procedure for Determinations by Inductively Coupled Plasma

- Step 1 - Set up the instrument as recommended by the manufacturer. The instrument must be allowed to become thermally stable before analysis begins (10 to 30 minutes).
- Step 2 - Profile and calibrate the instrument according to the recommended procedures of the manufacturer. Flush the system with the calibration blank between each standard.
- Step 3 - Begin sample analysis, flushing the system with the method blank solution or DI water between each sample.
- Step 4 - Dilute with 1:1 extract:DI water solution and reanalyze any samples for which the concentration exceeds the calibration range, and report aliquot and diluted volumes.
- Step 5 - Record results for Ca^{2+} ; Mg^{2+} ; Na^{+} ; and Al^{3+} . Analyze K^{+} by AAS or ES.

Procedure for Determinations by Emission Spectroscopy

NOTE: Locate instrument in an area away from direct sunlight and in an area free of drafts, dust, and tobacco smoke. Guard against contamination from corks, filter paper or pulp, perspiration, soap, cleansers, cleaning mixtures, and inadequately rinsed apparatus. Because of differences among instruments, it is impossible to formulate detailed operating instructions. Follow recommendations of the manufacturer for selecting proper photocell and wavelength, for adjusting slit width and sensitivity, for appropriate fuel and oxidant pressures, and for the steps for warm-up, correcting for interferences and flame background, rinsing of burner, igniting sample, and measuring emission intensity.

- Step 1 - Calibrate the instrument.
- Step 2 - Analyze the samples.

- Step 3 - Dilute with 1:1 extract:DI water solution and reanalyze any samples for which the concentration exceeds the calibration range and record aliquot and diluted volumes.
- Step 4 - Record results for K^+ and Na^+ . Analyze Ca^{2+} and Mg^{2+} by ICP or AAS and Al^{3+} by ICP.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
 QCCS, Samples 9 to 16, MB, DUP,
 QCCS, Samples 16 to 25, SRM,
 QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
 MB = method blank
 QCPS = quality control preparation sample
 DUP = duplicate sample
 SRM = standard reference material

Calculations and Reporting

The appropriate reporting units for the exchangeable cations are meq/100 g of soil.

Final Extract Volume = [Final wt. (g) + Tare wt. (g)] ÷ 1.0105

NOTE: data for this calculation is obtained during the determination of the cation exchange capacity. 1.0105 is the density of the NH_4Cl extraction solution.

Exchangeable Cation (meq/100 g) =

$\{[(\text{inst. reading in mg/L}) \times (\text{dilution factor in mL/mL, if applicable})] \times (\text{final extract vol. in mL}) \times (1 \text{ L} \div 1000 \text{ mL}) \times (\text{meq} \div \text{atomic weight in mg})\} + [(\text{sample weight in mg}) \times (\text{moisture correction, if applicable})] \times 100$

NOTE: moisture corrections are applicable if sample extracted is not oven-dried, thereby, removing all free water. The moisture correction factor is as follows:

$[(1 - \text{moisture content in \%}) \div (100 + \text{moisture content in \%})]$

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Amorphous Iron and Aluminum Oxides

Introduction

The differentiation of amorphous and finely divided crystalline oxides of Fe, and to a lesser extent Al, has been used for the past two decades in studies related to soil genesis and profile development (McKeague and Day, 1966; Blume and Schwertmann, 1969; Alexander, 1974). The ratio of amorphous to crystalline oxides is thought to decrease with increasing soil age. Recent research, on the effects of acid precipitation on soils, has provided contradictory evidence as to the importance of amorphous Fe and Al oxides. Some authors noted that this amorphous phase is responsible for most of the SO_4 adsorption (Parfitt and Smart, 1978; Rajan, 1978; Singh, 1980). Johnson and Todd (1983) found, however, that only the crystalline Fe oxides correlate significantly with SO_4 adsorption. Part of this discrepancy may be because under certain soil conditions, the extractant used is not specific for the removal of amorphous Fe and Al oxides.

Review of Methods

For the past twenty years, the most commonly used method for amorphous Fe and Al oxides removal from soils has been extraction with acid ammonium oxalate (NH_4Ox) as described by Schwertmann (1959). Schwertmann (1964, 1973) found that the extraction must be performed in the dark, as UV light causes varying degrees crystalline Fe oxides dissolution. Without UV light only the amorphous fraction is extracted. Similarly, Iyengar et al., (1981) reports that for Virginia soils, NH_4Ox extraction in the dark removed lower levels of Fe and Al than citrate-dithionite (CD) or NH_4Ox under UV light, and had little effect on the X-ray diffractive mineralogy of the soils. Since NH_4Ox removes amorphous organic and inorganic Fe and Al, this method has most often been used in conjunction with Na-pyrophosphate treatment (to remove organic Fe and Al) in order to isolate the amorphous inorganic fraction.

Although widely used, problems have been reported with this method. Baril and Britton (1967) found that larger amounts of Fe were recovered by NH_4Ox than CD in some coarse textured magnetite-containing soils from Quebec. Later, Gamble and Daniels (1972) showed that magnetite was sparingly soluble in citrate-bicarbonate-dithionite but quite soluble in NH_4Ox . More recently, Rhoton et al., (1981) and Walker (1983) found that the magnetite dissolution by NH_4Ox overestimates the amorphous Fe in soil and sediment samples. Consequently, Walker (1983) suggests magnetite removal from the sample prior NH_4Ox treatment, and Rhoton et al., (1981) suggest that caution should also be exercised if siderite and/or pyrite are present.

Similar problems have also been encountered with aluminum. Johnson and Todd (1983) report higher levels of NH_4Ox -extractable Al than CD-extractable Al in soils from New Hampshire and Washington. The mineral horizons of soils from Central Ontario have shown similar trends (Ontario Ministry of the Environment, unpublished data). McKeague and Day (1966) report the removal of interlayer Al from artificially prepared Al-chloritized bentonite during a four hour NH_4Ox treatment in the dark. They also noted, however, that this treatment only slightly attacked the interlayers of natural Al-chloritized clays.

It is evident that the success of NH_4Ox for the removal of amorphous Fe and Al will depend largely on the type and mineralogy of the soil. Recently, Chao and Zhou (1983) found that acid hydroxylamine dissolved virtually no magnetite and recommended further testing of this reagent as an extractant for soils. Ross et al., (1985) expressed that the low pH of this solution (pH 1) would effect Fe-chlorite. These authors tested acid hydroxylamine on chlorite clay minerals as well as on other widely varying soil types, including the Agriculture Canada round-robin ECSS samples. The hydroxylamine did not appear to increase Fe-chlorite dissolution markedly and for the remaining

samples, NH_4Ox -extractable Fe exceeded hydroxylamine extractable Fe in half the cases. Some of these could be explained by the presence of magnetite. The data, however, also indicates that for every sample ($n=27$), hydroxylamine extracts more Al than NH_4Ox , in some cases up to five times more. Although these authors conclude that overnight hydroxylamine extraction can be used as an alternative method for the removal of amorphous Fe and Al species, it is felt that this reagent requires further investigation before it is used routinely.

Reference Method

The above evidence suggests that NH_4Ox is not necessarily specific for the removal of amorphous Fe and Al species from certain soil types. Other reagents should be tested for this purpose. We, therefore, do not suggest the routine measurement of the amorphous Fe and Al fractions on soil samples connected with LRTAP terrestrial monitoring programs. On a smaller scale, perhaps where the soil mineralogy is known, or in LRTAP research programs, the measurement of this fraction might be feasible. In these cases, the reference procedure is outlined by Agriculture Canada (1984).

The NH_4Ox extraction at pH 3.0 should be performed for 4 hours in the dark using a soil:solution ratio of 1:40. Fe and Al levels have been found to vary with soil:solution ratio (Ross et al., 1985) and 1:40 is the more commonly used ratio. The results provided by this method should be interpreted with caution keeping in mind the inherent limitations of the technique.

Work by the Ontario Ministry of the Environment has shown, particularly for Al, that the amount extracted is dependent on sample grinding. If less than a one gram aliquot is used for extraction, it is suggested that in order to obtain a representative sample, a soil sample ground to less than 0.5 mm be used. This will minimize enhanced Al results due to grinding. It is advisable to sieve out the naturally less than 0.5 mm fraction first and then grind the remaining sample to pass through a 0.5 mm (35 mesh) sieve. Overgrinding must be avoided. For further information, refer to Neary and Barnes (1993).

Summary of Method

Soil samples are extracted for four hours with acid ammonium oxalate. The oxalate solution reduces Fe and Al to lower valence states allowing their removal from the soil. The extraction must be performed in the dark as ultraviolet light has been found to promote the dissolution of crystalline iron and aluminum interlayer substances (Iyengar et al., 1981; Schwertmann, 1964). Samples are centrifuged and the supernatant analyzed for Fe and Al by atomic absorption spectrophotometry or by inductively coupled plasma atomic emission spectroscopy.

Interferences and Shortcomings

It has been suggested that acid ammonium oxalate can remove some crystalline forms of Fe and Al, therefore, the procedure may not be specific for amorphous Fe and Al in some soil types.

Due to high levels of dissolved solids in the extracts, aspirator clogging and residue build-up on the burner head may occur. This may be alleviated somewhat by the aspiration of large amounts of distilled water between samples. Dilution of samples may also be necessary.

NOTE: If dilution with deionized (DI) water is used, matrix-matched calibration standards and QC solutions must also be diluted.

Safety

Normal laboratory safety precautions should be observed. A face shield, apron and gloves should be worn when preparing the acid ammonium oxalate solution. Standard safety precautions should be followed when analyzing samples using a nitrous oxide/acetylene flame.

Apparatus and Equipment

- tubes, polystyrene, disposable, 17 X 100 mm, with caps, 15 mL.
- dispenser, Oxford or equivalent, capable of dispensing 10 mL liquid.
- glazed weighing paper.
- flasks, volumetric, 1 L.
- filter paper, Whatman #1.
- shaker, reciprocating, Eberbach or equivalent.
- centrifuge, capable of holding 17 X 100 mm, 15 mL tubes, and reaching 2500 rpm.
- pH meter, and electrode.
- balance, accurate to 0.001 g.
- tubes to fit AAS or ICP autosampler.

Reagents and Consumable Materials

- ammonium oxalate, $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$, crystals, reagent grade.
- oxalic acid, $\text{H}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$, crystals, reagent grade.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- acid ammonium oxalate solution--In a 1 L volumetric flask, dissolve 16.06 g $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ and 10.97 g $\text{H}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ in DI water. Adjust pH to 3.0 by adding either ammonium oxalate or oxalic acid crystals. Dilute to 1 L with DI water.

Calibration and Standardization

Within each class of instruments (AAS and ICP), the calibration procedure varies slightly. Calibrate by analyzing a calibration blank (0 mg/L standard) and a series of at least three additional standards within the linear range of the instrument. If an ICP is used, a multi-element standard may be prepared and analyzed. For AAS determinations, the instrument must be calibrated for each analyte by using a separate standard. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded. The calibration should be updated every four hours when running the instrument.

Prepare calibration standards so that the final concentration of NH_4Ox in the standards is equal to that of the sample extracts. It is advisable to have two quality control solutions, one in the high and one in the low part of the operating range.

To correctly set-up the AAS, refer to the operating manual for the instrument. Optimal wavelengths are: 309.3 nm for Al and 248.3 nm for Fe. A nitrous oxide/acetylene flame is used for Al and an air/acetylene flame is used for Fe.

Procedure

Step 1 - Onto tared, glazed weighing paper, weigh 0.250 g of air-dried soil, ground to pass through a 0.5 mm (35 mesh) sieve.

NOTE: It is advisable to sieve out the naturally less than 0.5 mm fraction first, and then grind the remaining sample aliquot (with frequent resieving) to pass through the sieve. This eliminates undue grinding of the fraction which is already less than 0.5 mm and helps to prevent enhanced Al results.

Step 2 - Transfer soil to a labelled, 15 mL disposable tube.

Step 3 - Using the dispenser, add 10 mL acid ammonium oxalate solution to each tube.

Step 4 - Cap tubes and shake at low speed on a reciprocating shaker for four hours in the dark.

Step 5 - Remove tubes from shaker and centrifuge at 2500 rpm until clear.

Step 6 - Decant supernatant into clean, labelled disposable tubes. Filter samples if sediment is present.

Step 7 - Analyze samples by AAS or ICP.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be

less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

If a chart recorder is used, peak heights are measured in mm from a baseline drawn between peaks. The Fe and Al concentrations are calculated, taking into account sensitivity changes throughout the run, blank values and dilution factors. Results are reported as % Fe and Al in the soil. Results are reported to two significant figures.

$$\text{Fe (\%)} = \frac{\text{Fe } (\mu\text{g/mL}) \text{ in solution} \times 10 \text{ mL}}{(250 \text{ mg soil} \times 1000 \mu\text{g/mg})} \times 100$$

$$\text{Al (\%)} = \frac{\text{Al } (\mu\text{g/mL}) \text{ in solution} \times 10 \text{ mL}}{(250 \text{ mg soil} \times 1000 \mu\text{g/mg})} \times 100$$

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Organic Iron and Aluminum

Introduction

Micronutrient chemistry in the terrestrial environment largely involves complexation reactions with organic substances. Much research has involved the isolation of soil humic substances and their associated micronutrients, particularly Fe and Al. Humic and fulvic acids are able to form stable complexes with metal ions as a result of their high levels of oxygen-containing functional groups. Organic Fe and Al complexes accumulate in the mineral horizons of certain types of soils and can be used to distinguish podzolic (spodic) B horizons. It is likely that the organic binding of metals, such as Fe and Al, plays a significant role in controlling potentially toxic levels of these elements in the soil and drainage waters. Indeed, micronutrient cations in displaced soil solutions have been found to occur partly in organically bound forms (Geering et al., 1969). With mounting evidence to demonstrate higher aluminum solubility with watershed acidification, the proportion of Fe and Al bound by organics may be important information in LRTAP terrestrial monitoring programs.

Through various extraction procedures, an approximate differentiation can be made between organic Fe and Al and other secondary accumulation products, such as Fe and Al oxides. Extractants for Fe and Al oxides also remove organic forms of these elements. This means that a separate extraction to remove organic Fe and Al is required before the oxide fraction can be isolated. This distinction may be of particular importance in LRTAP terrestrial studies where amorphous and finely divided crystalline oxide fraction have been shown to largely control SO_4^{2-} adsorption (Chao et al., 1962; Rajan, 1978; Neary et al., 1987). In turn, soil cation leaching can be controlled by SO_4^{2-} adsorption (Huete and McColl, 1984). Organic matter has been found by some researchers to have a negative influence on SO_4^{2-} adsorption (Johnson and Todd, 1983).

For the reasons outlined above, it is suggested that organically bound Fe and Al in soils be measured in LRTAP terrestrial monitoring studies. The amount of organic Fe + Al in the B horizons of soils has also been incorporated into the Canadian and American soil classification systems. If a terrestrial monitoring program is to include the soil classification and the identification of groups of soils susceptible to acidification, the measurement of this fraction is necessary.

Since this is expected to be a relatively stable parameter, this measurement need only be done initially to help characterize the soil.

Review of Methods

The search for an ideal extractant stemmed primarily from efforts to find a satisfactory reagent which would remove humic substances from the soil, yet allow characterization of these substances. A necessary requirement, therefore, has been that the extractant not alter the physical and chemical properties of the organic material. Strongly alkaline solutions, organic acids, and neutral salt solutions have all been tested. The major criticism against using caustic alkali has been

the possible modification of the extracted humic substances, although some evidence has been presented to refute this argument. Smith and Lorimer (1964) found that the fulvic acid extracted by NaOH had a similar composition to that extracted by water. Similarly, Schnitzer and Skinner (1968) found that the fulvic acid removed by 0.5 N NaOH and 0.1 N HCl had similar elemental compositions. Hayes (1985), on the other hand, found that strong alkali and associated chemical oxidation destroyed organic matter.

Neutral salts of mineral or organic acids have also been used, however, their success depends upon the ability of their anions to interact with the cations in combination with humic material in the soil. Bremner and Lees (1949) tested the sodium salts of eight inorganic acids, ten organic acids as well as NaOH and NaCO₃. They concluded that Na-pyrophosphate (Na₄P₂O₇) was the best in providing a mild but reasonably efficient extractant. They recommend a concentration of at least 0.1 M. Below this minimum concentration, yield varies with concentration and results in decreased efficiency. Schnitzer et al., (1958) also found that Na₄P₂O₇ (as well as Na₂CO₃, Na₃PO₄, NaOH, HF and Na-EDTA) removed more than 80% of the organic material from a Bh horizon.

Aleksandrova (1960) was the first to show that Na₄P₂O₇ can extract not only humic substances, but also organo-mineral complexes without destroying non-silicate forms of the sesquioxides. Later, Bascomb (1968) found that 0.1 M K₄P₂O₇ extracted Fe and amorphous gel hydrous oxides but not amorphous aged hydrous oxides. A comparison of Na₄P₂O₇ with oxalate and Na₄P₂O₇-dithionite indicated that Na₄P₂O₇ was the most specific for organically complexed Fe and Al (McKeague, 1967). McKeague et al., (1971) report that although reasonably specific for organic Fe, 0.1 M Na₄P₂O₇ is less specific for Al as shown by its ability to also remove Al from an Al-hydroxide sol. These authors concluded that this extractant can be used for the approximate distinction of organic Fe and Al in soils. Recent work at the Ontario Ministry of the Environment has found a much better correlation between organic C and Na₄P₂O₇-extractable Fe than Al even after high speed centrifugation of sample extracts. The above evidence suggests that the Fe and Al measured in a pyrophosphate extract cannot be designated as only organic, although much of it is probably in an organic form.

Later research has shown that many of the problems identified above resulted from the methods used to clarify the solution prior to instrument analysis. McKeague (1967) reported that unbuffered Na₄P₂O₇ (pH approximately 10) mainly attacks organic Fe and Al, but that high speed centrifugation (20,000 rpm) after extraction is required to remove suspended inorganic Fe and Al. He presented evidence to show that centrifugation at 20,000 rpm lowers Fe and Al levels but not C levels. Subsequent work showed decreasing Fe and Al levels with increasing centrifuging times and speeds (McKeague and Schuppli, 1982). This was attributed to suspended material (e.g., colloidal material or polymerized pyrophosphate complexes forming after extraction). These findings demonstrate the need for an established centrifuging procedure.

Jeanroy and Guillet (1981) suggest that a portion of the suspended material may be peptized amorphous hydrous oxides. Ballantyne et al., (1980) report that additional amounts of Superfloc progressively decrease the Fe and Al concentration in the extracts. Sheldrick and McKeague (1975) compared high speed centrifugation with the addition of 0.5 mL of 0.1% Superfloc + low speed centrifugation. They concluded that the methods give similar results although the Superfloc values

were commonly slightly higher. Schuppli et al., (1983) experimented on Canadian and tropical soils using high speed centrifugation, Superfloc + low speed centrifugation, and both of the above with ultrafiltration. In the tropical soils, Superfloc + low speed centrifuging removed all the Al silicates but concentrated goethite in the remaining suspended material. Similar results were obtained with high speed centrifugation but with less goethite. This effect was not nearly as pronounced in the Canadian soils. They concluded that high speed centrifugation or low speed centrifugation + Superfloc alone (without ultrafiltration) was adequate for most temperate region soils. Ultrafiltration seems most suited to laboratories not equipped with a high speed centrifuge. To date, the form of Fe and Al remaining in ultracentrifuged or ultrafiltered samples has not been adequately characterized.

The pH of the extracting solution has been of some concern to analysts. If maintaining the integrity of the humic material is necessary for the identification of the organics, an alkaline solution, such as unbuffered $\text{Na}_4\text{P}_2\text{O}_7$, may not be suitable. Bremner and Lees (1949) speculated that in order to decrease the potential alteration of proteins, the pH of the extractant should be 7. Data compiled from a series of studies shows the pH 7 method to be superior for minimizing the alteration of humic substances (Hayes, 1985). Kononova (1966) also recognized the need to adjust the pH to 7 but recommended that because the efficiency of the extractant, for humic acids especially, is better at a higher pH, a pH of 9 should be used. Certainly, if one's primary interest is the measurement of organic Fe and Al, rather than the characterization of the organics, a higher pH is probably suitable. A comparison performed by the Ontario Ministry of the Environment found that the Fe and Al extracted at pH 10 with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ correlates better with organic C than 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ at pH 7 or 0.01 M $\text{Na}_4\text{P}_2\text{O}_7$ at pH 7.

Adopted standard methods in Canada, the U.K, and the U.S. involve the use of 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ at pH 9.6 or 10 with either high speed centrifugation or the addition of Superfloc + low speed centrifugation. Of these methods, Loveland and Digby (1984) report that $\text{Na}_4\text{P}_2\text{O}_7$ at pH 10 with high speed centrifugation (20,000 rpm) is a more consistent treatment, particularly for Al.

The Canadian and U.K. methods use samples ground to less than 0.5 mm whereas the U.S. EPA methods (Blume et al., 1990) use a larger sample aliquot and the less than 2 mm sample. In order to obtain a representative sample, it is suggested that if less than one gram of sample is chosen for extraction, the sample should be ground to less than 0.5 mm prior to extraction. Overgrinding must be avoided. The extracts may be analyzed by either atomic absorption spectrophotometry (AAS) or by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Reference Method

The reference extraction method for the removal of organically-bound Fe and Al from soil is treatment with 0.1M $\text{Na}_4\text{P}_2\text{O}_7$ unbuffered or at pH 10. Centrifuging at 20,000 rpm is recommended, although Superfloc + low speed centrifugation (with ultrafiltration optional) may be used, as it is recognized that not all laboratories are equipped with a high speed centrifuge. This method was chosen for the following reasons:

- the efficiency of the alkaline extraction for humic material,
- identification of the humic substances is not required,
- the results of previous work indicating its usefulness for the approximate differentiation of organic Fe and Al in soils (McKeague et al., 1971) and the identification of podzolic (spodic) B horizons, and
- Adoption of this method by the Canadian, USDA, and British soil classification systems.

An overnight extraction (shaking) period is suggested since Bremner and Lees (1949) found that after 18 to 20 hours the amount of humic material extracted (as measured by percent N) does not increase.

Summary of Method

A sample of air-dried soil, ground to less than 0.5 mm, is weighed into a plastic centrifuge tube. Sodium pyrophosphate solution (0.1 M at pH 10) is added, the tube is capped and shaken on a reciprocating or end-over-end shaker at low speed overnight (18-20 hrs). The tube is centrifuged at high speed for 15 minutes. Alternatively, 0.5 mL of 0.1% Superfloc is added and the tube is centrifuged at low speed for 15 to 30 minutes or until solutions are clear. Turbid samples should be filtered through a 0.10 μ m membrane filter. Iron and aluminum are determined in the supernatant by AAS or ICP.

Interferences and Shortcomings

When analyzing extracts by AAS, aspirator clogging and residue build-up on the burner head may occur. This may be alleviated somewhat by the aspiration of large amounts of deionized water (DI) between samples. Alternatively, samples may be diluted prior to analysis (Fe and Al levels permitting), analyzed using micro-sampling (available on some instruments), or digested with HNO_3 and H_2SO_4 prior to analysis.

NOTE: If dilution with DI water is used, matrix-matched calibration standards and QC solutions must also be diluted.

A high bias in results will occur if samples are turbid. High speed centrifuging or the addition of Superfloc and low speed centrifuging is required to obtain extracts which are free of suspended material.

Although it is known that a large amount of the Fe and Al extracted by this method is in the organic form, it is possible that other forms of iron and aluminum may be extracted by this method. The results can, therefore, only be used for the approximate differentiation of organic Fe and Al in the soil.

Safety

No specific hazards are associated with this procedure or with the required reagents. Normal laboratory safety practices are to be observed. Protective clothing and safety glasses should be worn when handling concentrated HNO_3 or H_3PO_4 or NaOH . Standard safety precautions should be observed when analyzing samples using a nitrous oxide/acetylene flame.

Apparatus and Equipment

- centrifuge tubes, plastic 50 mL, Oak Ridge type, screw closure.
- screw caps to fit centrifuge tubes.
- tube rack to hold centrifuge tubes.
- tubes to fit AAS or ICP autosampler.
- spatula.
- glazed weighing paper.
- dispenser, capable of accurately dispensing 30 mL of liquid.
- reciprocating shaker, Eberbach, end-over-end shaker, or equivalent.
- centrifuge with head to hold 50 mL tubes (preferably capable of attaining a speed of 20,000 rpm or 510 X G. This is not mandatory, as a speed of 2000 rpm may be used with the addition of Superfloc).
- balance, accurate to 0.001 g.
- pH meter.
- 0.1 μm membrane filters and filter apparatus (optional).

Reagents and Consumable Materials

- sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$), anhydrous reagent grade powder or $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$.
- nitric acid (HNO_3), concentrated, reagent grade.

- Superfloc (N-100) only required if a high speed centrifuge is not available. Two known sources for this product are: Cyanamid of Canada, Montreal, Quebec or from the American Cyanamid Co. of Wayne, New Jersey, U.S.A.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- sodium hydroxide (NaOH), 0.1 M solution or phosphoric acid (H_3PO_4) as required to adjust the pH of the pyrophosphate to 10.
- sodium pyrophosphate solution, 0.1 M--Into a one litre volumetric flask, weigh out 26.59 g anhydrous $Na_4P_2O_7$, oven dried and cooled in a desiccator (44.61 g if using $Na_4P_2O_7 \cdot 10H_2O$). Dissolve in DI water and dilute to one litre. Adjust to pH 10 with NaOH or H_3PO_4 .

Calibration and Standardization

Within each class of instruments (AAS and ICP), the calibration procedure varies slightly. Calibrate by analyzing a calibration blank (0 mg/L standard) and a series of at least three additional standards within the linear range of the instrument. If an ICP is used, a multi-element standard may be prepared and analyzed. For AAS determinations, the instrument must be calibrated for each analyte by using a separate standard. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded.

Prepare calibration standards so that the final concentration of $Na_4P_2O_7$ in the standards is equal to that of the sample extracts. It is advisable to have two quality control solutions, one in the high and one in the low part of the operating range.

To correctly set-up the AAS, refer to the operating manual for the instrument. Optimal wavelengths are: 309.3 nm for Al and 248.3 nm for Fe. A nitrous oxide/acetylene flame is used for Al and an air/acetylene flame is used for Fe.

Procedure

Step 1 - Onto tared, glazed weighing paper, weigh 0.300 g of air-dried soil, ground to pass through a 0.5 mm (35 mesh) sieve.

NOTE: It is advisable to sieve out the naturally less than 0.5 mm fraction first and then grind the remaining sample aliquot to pass through the 0.5 mm sieve. This eliminates undue grinding of the naturally less than 0.5 sample and helps prevent enhanced Al results (Neary and Barnes, 1993).

Step 2 - Transfer into a labelled 50 mL plastic centrifuge tube.

- Step 3 - Using the dispenser, add 30 mL of 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ extracting solution to each tube.
- Step 4 - Cap tubes and shake overnight on a reciprocating shaker at low speed or on an end-over-end shaker at 40 to 50 rpm.
- Step 5 - Remove tubes from shaker. Uncap tubes and centrifuge at 20,000 rpm for 15 minutes. Alternatively, add 0.5 mL of 0.1% Superfloc solution to the sample and centrifuge at 1500 for 15 to 30 minutes or until clear. Filter any turbid samples through 0.1 μm membrane filters.
- Step 6 - Carefully decant supernatant into labelled 15 mL disposable tubes. Acidify extracts with 3 drops of concentrated nitric acid and let samples sit overnight allowing any suspended matter to settle.
- Step 7 - Analyze for Fe and Al by AAS or ICP.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Between laboratory precision can be determined by analyzing the Agriculture Canada ECSS round-robin samples.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

If a chart recorder is used, peak heights are measured in mm from a baseline drawn between peaks. Calculate Fe and Al concentrations, accommodating for sensitivity changes throughout the run, blank values and dilution factors. Results are reported as % Fe and Al in the soil. Results are reported to two significant figures.

$$\text{Fe (\%)} = \frac{\text{Fe (\mu g/mL) in solution} \times 30 \text{ mL} \times 100}{(300 \text{ mg soil} \times 1000 \text{ }\mu\text{g/mg})}$$

$$\text{Al (\%)} = \frac{\text{Al } (\mu\text{g/mL}) \text{ in solution} \times 30 \text{ mL}}{(300 \text{ mg soil} \times 1000 \mu\text{g/mg})} \times 100$$

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Iron and Aluminum Oxides

Introduction

The accumulation of amorphous and finely divided crystalline sesquioxides in soil B horizons have often been examined in soil dating, podzolization and weathering studies. It is generally believed that the degree of crystallinity of Fe and Al oxide weathering products increases with the age of the soil. Recent work has shown that these secondary oxides play an important role in the adsorption of SO_4^{2-} by the soil (Chao et al., 1964; Rajan, 1978; Johnson and Todd, 1983; Singh, 1984; Neary et al., 1987). Moreover, inorganic Al oxide phases are believed to be the source of the high inorganic monomeric Al levels found in soil solutions from the B horizons of podzols (Lazerte, 1987). The partitioning of secondary iron and aluminum oxides may, therefore, be of significance in LRTAP terrestrial monitoring studies. Since this is expected to be a relatively stable parameter, this measurement need only be done once to help characterize the soil.

Review of Methods

A satisfactory extractant for the removal of secondary Fe and Al oxides from soils must efficiently recover the sesquioxide fraction without solubilizing Fe and Al from primary and clay minerals. Aguilera and Jackson (1953) proposed the use of sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) with sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), adjusted to pH 7.3 with 10% sodium hydroxide (NaOH), for the rapid and almost complete removal of free Fe oxides from soils (as well as the removal of organically bound Fe). Jackson (1956) later modified the method and added the $\text{Na}_2\text{S}_2\text{O}_4$ as a powder without the addition of NaOH . Unfortunately, this prevented the precipitation of FeS at low pH levels and resulted in the ineffective removal of Fe oxides due to a low oxidation potential. Mehra and Jackson (1960) overcame these problems by stabilizing the pH at 7.3 with sodium bicarbonate (NaHCO_3) as a source of hydroxyl ions. A pH of 7.3 was chosen because the oxidation potential of $\text{Na}_2\text{S}_2\text{O}_4$ - $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ systems buffered with NaHCO_3 increases rapidly up to pH 8, while the solubility of Fe_2O_3 decreases rapidly over pH 7. The two curves intersect at about pH 7.3 suggesting that this is the optimum pH for extraction.

The Soil Research Institute of Agriculture Canada adopted Mehra and Jackson's (1960) method as their standard procedure. Sheldrick and McKeague (1975) in a methods comparison study found that for 14 Canadian soil samples, the Fe and Al levels obtained by Mehra and Jackson's (1960) method were comparable to the method used by the U.S. Department of Agriculture's Soil Conservation Service (USDA-SCS). The USDA-SCS method involved overnight shaking with citrate-dithionite (CD) solution. In the early 1980's, Agriculture Canada adopted the USDA-SCS method as their standard procedure.

Another reagent which has been tested for the removal of Fe and Al oxides is acid ammonium oxalate. Schwertmann (1964) suggested that acid ammonium oxalate in U.V. light could be used to remove crystalline + amorphous Fe and Al oxides. However, Iyengar et al., (1981) reported that under U.V. light, ammonium oxalate caused substantial dissolution of Al from hydroxy-Al interlayered vermiculite. When citrate-dithionite was used no detectable alterations to the mineral phases were identified.

Sample extracts may be analyzed by either atomic absorption spectrophotometry (AAS) or inductively coupled plasma optical emission spectroscopy (ICP-OES). Work by Raad et al., (1969) has shown that although the extracts contain high levels of dissolved solids, it is not necessary to destroy the organic matter in the extract by acid digestion prior to analysis of the samples.

Reference Method

A citrate-dithionite (CD) treatment is preferred for the removal of free Fe and Al oxides (as well as the removal of organic Fe and Al) from soils. If the pyrophosphate-extractable Fe and Al fraction is subtracted from the CD-extractable Fe and Al, approximate measures of crystalline + amorphous Fe and Al oxides are obtained.

Based on the findings of Sheldrick and McKeague (1975), either the method of Mehra and Jackson (1960) or a method involving the overnight shaking of the CD treatment may be used. The latter is suggested as it is time efficient and it is more widely used in North America. It is also recommended that, regardless of the method chosen, the Agriculture Canada ECSS round-robin samples (or other samples with known amounts of CD-extractable Fe and Al) should be analyzed with each new batch of $\text{Na}_2\text{S}_2\text{O}_4$ used. The Ontario Ministry of the Environment has found that $\text{Na}_2\text{S}_2\text{O}_4$ has a limited shelf life and with time the reducing power of this reagent decreases causing poor recovery of Fe and Al from the samples.

The Canadian standard method uses sample ground to less than 0.5 mm, whereas the USDA-SCS uses a larger sample aliquot (4 g) and an unground <2 mm soil sample. In order to obtain a representative sample, it is suggested that if a sample aliquot of less than 1 gram is chosen for extraction, a sample ground to less than 0.5 mm sample should be used. Overgrinding must be avoided (Neary and Barnes, 1993).

The sample extracts may be analyzed for Fe and Al by either ICP or AAS.

Summary of Method

A sample of air-dried soil, ground to less than 0.5 mm, is weighed into a plastic centrifuge tube. Sodium citrate solution (0.68 M) and $\text{Na}_2\text{S}_2\text{O}_4$ powder is added to the tube. The tube is capped and shaken overnight on either an end-over-end or reciprocating shaker. The samples are then centrifuged, either at low speed with the addition of Superfloc or at high speed without a flocculating agent. The supernatant is decanted and the extracts are analyzed by either AAS or ICP.

Interferences and Shortcomings

Due to the high levels of dissolved solids in the extracts, aspirator clogging and residue build-up on the burner head may occur. The aspiration of large amounts of deionized (DI) water between samples will alleviate this problem. Alternatively, some AAS instruments have a microsampling option which helps prevent clogging and residue build-up. Samples may also be digested with HNO_3 and H_2SO_4 to destroy the organics. The samples may be diluted (Fe and Al levels permitting).

NOTE: If dilution with DI water is used, matrix-matched calibration standards and QC solutions must also be diluted.

It is necessary that extracts are free of turbidity. The filtration of samples which remain turbid after centrifugation is recommended. After dilution and mixing of the sample extracts, samples should be left to sit overnight before analysis, to allow any suspended particles to settle.

The potential removal of Fe and Al from silicates by the citrate-dithionite extraction is generally believed to be negligible. However, it should be noted that this extractant also removes organic Fe and Al. If only Fe and Al oxides content is desired, the pyrophosphate-extractable Fe and Al concentrations must be analyzed and subtracted from the results of this analysis. In this case, the results are also subject to the inherent limitations of the pyrophosphate technique.

Safety

No specific hazards are associated with this procedure or with the required reagents. Normal laboratory safety practices are to be observed. Protective clothing and safety glasses should be worn when handling concentrated HNO_3 . Standard safety procedures should be followed when analyzing samples using a nitrous oxide/acetylene flame.

Apparatus and Equipment

- plastic centrifuge tubes, 50 mL, with screw caps.
- dispenser, capable of accurately dispensing 25 mL.
- reciprocating shaker (Eberbach or equivalent) or end-over-end shaker (40-50 rpm).
- spatula.
- glazed weighing paper.
- tubes to fit AAS or ICP autosampler.
- tube racks to hold 50 mL centrifuge tubes and AAS autosampler tubes.
- balance, accurate to 0.001 g.

Reagents and Consumable Materials

- sodium dithionite (hydrosulphite) ($\text{Na}_2\text{S}_2\text{O}_4$), reagent grade.
- sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), reagent grade.
- nitric acid (HNO_3), concentrated, reagent grade.
- Superfloc (N-100) only required if a high speed centrifuge is not available. Two known sources for this product are: Cyanamid of Canada, Montreal, Quebec or American Cyanamid Co. of Wayne, New Jersey, U.S.A.
- water—DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- sodium citrate solution (0.68 M)—In a one litre volumetric flask, dissolve 200 grams of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ in DI water. Dilute to volume with DI water.

Calibration and Standardization

Within each class of instruments (AAS and ICP), the calibration procedure varies slightly. Calibrate by analyzing a calibration blank (0 mg/L standard) and a series of at least three additional standards within the linear range of the instrument. If an ICP is used, a multi-element standard may be prepared and analyzed. For AAS determinations, the instrument must be calibrated for each

analyte by using a separate standard. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded.

Prepare calibration standards so that the final concentration of CD in the standards is equal to that of the sample extracts. It is advisable to have two quality control solutions, one in the high and one in the low part of the operating range.

To correctly set-up the AAS, refer to the operating manual for the instrument. Optimal wavelengths are: 309.3 nm for Al and 248.3 nm for Fe. A nitrous oxide/acetylene flame is used for Al and an air/acetylene flame is used for Fe.

Procedure

Step 1 - Onto tared, glazed weighing paper, weigh 0.500 g of air-dried soil, ground to pass through a 0.5 mm (35 mesh) sieve.

NOTE: It is advisable to sieve out the naturally less than 0.5 mm fraction first, and then grind the remaining sample aliquot to pass through the sieve. This eliminates undue grinding of the fraction which is already less than 0.5 mm and helps to prevent enhanced Al results.

Step 2 - Transfer weighed sample to a labelled 50 mL plastic centrifuge tube.

Step 3 - Using the dispenser, add 25 mL of 0.68 M sodium citrate solution.

Step 4 - Using a calibrated scoop, add 0.4 g of sodium dithionite.

Step 5 - Cap tubes and shake overnight on a reciprocating shaker at low speed, or on an end-over-end shaker (40-50 rpm).

Step 6 - Remove caps and centrifuge tubes for 15 minutes at 20,000 rpm. Alternatively, add 0.5 mL of 0.1% Superfloc solution to the sample and centrifuge at 1900 rpm for 30 minutes.

Step 7 - Carefully decant the supernatant into tubes to fit autosampler of the AAS. Acidify extracts with 3 drops of concentrated nitric acid and let samples sit overnight to allow any suspended material to settle.

Step 8 - Prepare a 1:5 dilution (1 mL sample and 4 mL of DI water) of the samples to give a convenient range for analysis.

NOTE: If samples are run without prior dilution, or if samples and standards are diluted, the extracting reagent concentrations of the QC solutions and standards must match the final dilution of the samples and the Fe and Al concentrations must be in the appropriate range.

Step 9 - Analyze samples by AAS or ICP.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
 MB = method blank
 QCPS = quality control preparation sample
 DUP = duplicate sample
 SRM = standard reference material

Calculations and Reporting

If a chart recorder is used, peak heights are measured in mm from a baseline drawn between peaks. The Fe and Al concentrations are calculated, taking into account sensitivity changes throughout the run, blank values and dilution factors. Results are reported as % Fe and Al in the soil. Results are reported to two significant figures.

$$\text{Fe (\%)} = \frac{\text{Fe } (\mu\text{g/mL}) \text{ in solution} \times 25 \text{ mL}}{(500 \text{ mg soil} \times 1000 \mu\text{g/mg})} \times 5 \times 100$$

$$\text{Al (\%)} = \frac{\text{Al } (\mu\text{g/mL}) \text{ in solution} \times 25 \text{ mL}}{(500 \text{ mg soil} \times 1000 \mu\text{g/mg})} \times 5 \times 100$$

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Phosphate Extractable Sulphate

Introduction

Sulphur, in the form of sulphate (SO_4^{2-}), is the principal anion in acidic deposition in eastern North America. Concern over the long-range transport and deposition of SO_4^{2-} in precipitation has lead to increased monitoring of the sulphur status of soils. In most mineral soils, the majority of extractable S is in the SO_4^{2-} form. In contrast, in organic horizons up to 50% of the total extractable S may be organically-bound (Maynard et al., 1987). The ability of soils to adsorb sulphate is one of the principal factors affecting the rate and extent of soil and watershed response to acidic deposition. Quantification of existing pools of adsorbed sulphate on a soil, concurrent with measurements of sulphate adsorption capacity of that soil, provide useful information for understanding the status and for predicting the future response of the soil to acidic deposition. The measurement of extractable sulphate is, therefore, suggested for terrestrial monitoring programs, such as LRTAP.

Review of Methods

The preparation and storage of soils is important in the determination of extractable SO_4^{2-} in soils. Several studies have shown that drying significantly altered the SO_4^{2-} content of the soil, particularly in organic horizons (Peverill et al., 1975; David et al., 1982; Searle and Sparling, 1987). Moreover, storage of air-dried samples at room temperature (20 to 25° C) for between 12 and 78 weeks resulted in significant increases in SO_4^{2-} concentrations (Maynard et al., 1987; Searle and Sparling, 1987). The increased sulphate concentrations observed were not consistent among soils. For extractable SO_4^{2-} measurements in organic horizons to be meaningful, the test should be performed on field-moist samples. A representative sample can be obtained by mixing/homogenizing the moist organic horizon prior to analysis. The changes in SO_4^{2-} concentration in mineral soils caused by drying and storage were much less than organic soils. For this reason and because of the difficulty in obtaining a representative sample from moist samples, air-dried soils are recommended for the analysis of mineral soils.

Numerous extractants have been proposed for the removal of SO_4^{2-} from soils (Beaton et al., 1968; Tabatabai, 1982). Most of the experimental work has involved using mineral agricultural soils in an attempt to correlate extractable SO_4^{2-} with plant growth and S uptake. Many of the techniques used on agricultural soils have been adapted for use on forest soils.

Sulphate extraction methods in soils can be broken into two groups based on the fraction of SO_4^{2-} removed. The two groups include those extractants that remove readily soluble SO_4^{2-} and those extractants that remove readily soluble SO_4^{2-} plus adsorbed SO_4^{2-} . The extractants used to remove readily soluble SO_4^{2-} (H_2O and weak salt solutions, such as CaCl_2 or NH_4Cl), are preferred for organic and mineral soils containing no appreciable amounts of adsorbed SO_4^{2-} . In contrast,

phosphate extractants, generally employing a $\text{Ca}(\text{H}_2\text{PO}_4)_2$ solution (500 mg P/L), are recommended for soils containing sesquioxides, allophane, or kaolinitic clays which have the potential to adsorb SO_4^{2-} . The latter class of soils generally includes the mineral horizons of most forest soils.

Eight extractants (including H_2O , several weak salts, and $\text{Ca}(\text{H}_2\text{PO}_4)_2$) were evaluated for extractable SO_4^{2-} on five forest organic soil horizons (Maynard et al., 1987). The most consistent extractant for SO_4^{2-} was 0.01 M NH_4Cl (1:10 soil to solution ratio), although all the weak salt extractants removed similar amounts of SO_4^{2-} . Water as an extractant gave the most variable results. The phosphate extractant did not remove any more SO_4^{2-} in the organic soils and gave more variable results than the NH_4Cl extractant. The strong phosphate solution was also noted to reduce the life of the ion chromatography column.

A phosphate extractant is preferred for mineral soils to ensure that strongly adsorbed SO_4^{2-} is extracted. Calcium dihydrogen phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) solution, containing 500 mg P/L, is preferred over Na or K phosphates since Ca enhances particle flocculation in clayey soils and makes filtering more convenient.

Over the last decade, the development of ion chromatography (IC) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have made the quantification of S and SO_4^{2-} rapid and more accurate. The determination of total S in a soil extract can be done by ICP-AES, but this technique is not specific for SO_4^{2-} . Ion chromatography is, however, specific to the SO_4^{2-} ion (Dick and Tabatabai, 1979; Nieto and Frankenberger, Jr., 1985; Maynard et al., 1987) and is, therefore, preferred for sulphate quantification in soil extracts.

Reference Method

The reference method for the extraction of the readily soluble SO_4^{2-} plus strongly adsorbed SO_4^{2-} phases from mineral soils employs a $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extraction solution containing 500 mg P/L. Sulphate in the extract is then quantified by ion chromatography. This method is primarily for use in the determination of extractable sulphate contents in mineral soil horizons but may be used for the analysis of organic horizons.

Summary of Method

Air-dried mineral horizons which have been disaggregated and passed through a 2-mm sieve are extracted with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ solution containing 500 mg P/L (2:20 air-dried soil to solution ratio). For organic horizons, the bulk sample is homogenized in the field-moist state prior to the removal of an aliquot for extraction with a $\text{Ca}(\text{H}_2\text{PO}_4)_2$ solution containing 500 mg P/L. Sulphate in the extracts is determined by suppressor or single column ion chromatography.

Interferences and Shortcomings

The high levels of phosphate in the $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extracts usually require adjustments to the eluent strength and instrument settings in order to provide a good separation between sulphate and phosphate. These adjustments may cause increased elution time for SO_4^{2-} . The column-life may also be shortened by the use of strong P solutions.

Colloidal material and certain organic compounds may interfere with the SO_4^{2-} peak on the chromatogram. Membrane filtering will help remove colloidal material and extend the life of the guard cartridges.

As with other soluble salts in soils, the amount of SO_4^{2-} extracted varies with the soil:extractant ratio. This ratio should, therefore, remain constant if samples are to be compared.

Safety

Wear protective clothing (laboratory coat and gloves) and safety glasses when preparing reagents. Follow the safety precautions of the manufacturer when operating instruments.

Apparatus and Equipment

- ion chromatograph, pump and conductivity detector. Either suppressed ion chromatography (SIC) such as the Dionex models, or single column ion chromatography (SCIC) such as the Waters systems.
- anion separator column and appropriate guard column.
- automated sampler and injection system and sample vials or tubes to fit sampler.
- data recording system, integrator or strip chart recorder.
- balance, accurate to 0.001 g.
- reciprocal shaker.
- vacuum filtration apparatus and funnels.
- vacuum membrane filter apparatus (optional).
- volumetric flask, 2 L.

- volumetric flask, 1 L
- Nalgene bottles, 60 mL

Reagents and Consumable Materials

- calcium dihydrogen phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$), reagent grade crystals.
- sodium sulphate (Na_2SO_4), reagent grade crystals.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extracting solution (500 mg P/L)--Weigh out 4.069 g $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ into a 2 L volumetric flask. Dissolve in DI water and dilute to 2 L volume.
- sulphate standard stock solution (1,000 mg SO_4/L)--Weigh 1.4790 g Na_2SO_4 (oven dried at 105° C and cooled in a desiccator) and transfer to a 1 L volumetric flask. Dissolve in $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extracting solution and dilute to one litre. Store refrigerated at 4° C.
- working sulphate standards prepared from the stock solution to cover expected range of SO_4^{2-} concentrations in sample extracts. Use $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extracting solution during dilution of the standard stock solution.
- appropriate eluent for the IC system and anion separator column used. (see manufacturer's recommendations).
- filter paper, Whatman #42.
- membrane filters, 0.45 μm (optional).

Calibration and Standardization

Follow the set-up procedures outlined in the manufacturer's operating manual for the specific instrument. Pump eluent through the system and stabilize the baseline. Adjust the recorder until zero is approximately 10% and the high standard is approximately 90% of the chart.

Use a minimum of three standards plus a zero standard to calibrate the system. The standards are analyzed and a calibration curve produced by plotting peak area or height against concentration. The concentration of standards should bracket the expected sample concentration;

however, the linear range of the instrument should not be exceeded. The calibration curve should be close to linear. Calibration standards should be prepared fresh daily. Calibration standards should be prepared in the extraction solution.

Procedure

- Step 1 - Weigh 2.00 g of air-dried soil into a 60-mL Nalgene bottle.
- Step 2 - Add 20 mL of $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extracting solution to each bottle.
- Step 3 - Shake for 1 h on a reciprocal shaker at 1 to 2 cycles per second.
- Step 4 - Vacuum filter the resulting suspension using Whatman #42 filter paper in a Buchner funnel.

NOTE: Alternately, samples may be centrifuged until the supernatant is clear.

- Step 5 - Vacuum filter the samples through 0.45 μm membrane filters.
- Step 6 - Analyze samples by ion chromatography within 24 hours.

NOTE: Samples may have to be transferred into vials or tubes specific to the type of sampler used.

NOTE: Samples should be stored at 4° C prior to sample analysis.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

The concentration of SO_4^{2-} is calculated by reading the peak area (or height) of the samples against the standard calibration curve. The calculation program used should take into account sensitivity changes throughout the run as detected by the in-run standard. All sample results are method blank subtracted.

For mineral soils, calculate extractable sulphate concentrations as follows:

$$\text{Extractable } \text{SO}_4^{2-} \text{ (mg S/kg)} = \frac{\text{SO}_4^{2-} \text{ (mg/L) in solution} \times \text{extract volume (mL)}}{\text{dry soil weight (g)}}$$

For organic samples, the sample weight must be based on % moisture in the field-moist sample. Therefore, the following formula is applicable:

$$\text{Extractable } \text{SO}_4^{2-} \text{ (mg S/kg)} = \frac{\text{SO}_4^{2-} \text{ (mg/L) in solution} \times \text{extract volume (mL)}}{(\text{initial sample weight (g)} \times \text{moisture correction})}$$

NOTE: The moisture correction factor is as follows with the moisture content presented as a fraction of the whole (e.g., 0.75):

$$[(1 - \text{moisture content in \%}) + (100 + \text{moisture content in \%})]$$

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Ammonium Chloride Extractable Sulphate

Introduction

Sulphur, in the form of sulphate (SO_4^{2-}), is the principal anion in acidic deposition in eastern North America. Concern over the long-range transport and deposition of SO_4^{2-} in precipitation has lead to increased monitoring of the sulphur status of soils. In most mineral soils, the majority of extractable S is in the SO_4^{2-} form. In contrast, in organic horizons up to 50% of the total extractable S may be organically-bound (Maynard et al., 1987). The ability of soils to adsorb sulphate is one of the principal factors affecting the rate and extent of soil and watershed response to acidic deposition. Quantification of existing pools of adsorbed sulphate on a soil, concurrent with measurements of sulphate adsorption capacity of that soil, provide useful information for understanding the status and for predicting the future response of the soil to acidic deposition. The measurement of extractable sulphate is, therefore, suggested for terrestrial monitoring programs, such as LRTAP.

Review of Methods

The preparation and storage of soils is important in the determination of extractable SO_4^{2-} in soils. Several studies have shown that drying significantly altered the SO_4^{2-} content of the soil, particularly in organic horizons (Peverill et al., 1975; David et al., 1982; Searle and Sparling, 1987). Moreover, storage of air-dried samples at room temperature (20 to 25° C) for between 12 and 78 weeks resulted in significant increases in SO_4^{2-} concentrations (Maynard et al., 1987; Searle and Sparling, 1987). The increased sulphate concentrations observed were not consistent among soils. For extractable SO_4^{2-} measurements in organic horizons to be meaningful, the test should be performed on field-moist samples. A representative sample can be obtained by mixing/homogenizing the moist organic horizon prior to analysis. The changes in SO_4^{2-} concentration in mineral soils caused by drying and storage were much less than organic soils. For this reason and because of the difficulty in obtaining a representative sample from moist samples, air-dried soils are recommended for the analysis of mineral soils.

Numerous extractants have been proposed for the removal of SO_4^{2-} from soils (Beaton et al., 1968; Tabatabai, 1982). Most of the experimental work has involved using mineral agricultural soils in an attempt to correlate extractable SO_4^{2-} with plant growth and S uptake. Many of the techniques used on agricultural soils have been adapted for use on forest soils.

Sulphate extraction methods in soils can be broken into two groups based on the fraction of SO_4^{2-} removed. The two groups include those extractants that remove readily soluble SO_4^{2-} and those extractants that remove readily soluble SO_4^{2-} plus adsorbed SO_4^{2-} . The extractants used to remove readily soluble SO_4^{2-} (H_2O and weak salt solutions, such as CaCl_2 or NH_4Cl), are preferred for organic and mineral soils containing no appreciable amounts of adsorbed SO_4^{2-} . In contrast,

phosphate extractants, generally employing a $\text{Ca}(\text{H}_2\text{PO}_4)_2$ solution (500 mg P/L), are recommended for soils containing sesquioxides, allophane, or kaolinitic clays which have the potential to adsorb SO_4^{2-} . The latter class of soils generally include the mineral horizons of most forest soils.

Eight extractants (including H_2O , several weak salts, and $\text{Ca}(\text{H}_2\text{PO}_4)_2$) were evaluated for extractable SO_4^{2-} on five forest organic soil horizons (Maynard et al., 1987). The most consistent extractant for SO_4^{2-} was 0.01 M NH_4Cl (1:10 soil to solution ratio), although all the weak salt extractants removed similar amounts of SO_4^{2-} . Water as an extractant gave the most variable results. The phosphate extractant did not remove any more SO_4^{2-} in the organic soils and gave more variable results than the NH_4Cl extractant. The strong phosphate solution was also noted to reduce the life of the ion chromatography column.

A phosphate extractant is preferred for mineral soils to ensure that strongly adsorbed SO_4^{2-} is extracted. Calcium dihydrogen phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) solution, containing 500 mg/L P, is preferred over Na or K phosphates since Ca enhances particle flocculation in clayey soils and makes filtering more convenient.

Over the last decade, the development of ion chromatography (IC) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have made the quantification of S and SO_4^{2-} rapid and more accurate. The determination of total S in a soil extract can be done by ICP-AES, but this technique is not specific for SO_4^{2-} . Ion chromatography is, however, specific to the SO_4^{2-} ion (Dick and Tabatabai, 1979; Nieto and Frankenberger, Jr., 1985; Maynard et al., 1987) and is, therefore, preferred for sulphate quantification in soil extracts.

Reference Method

The reference method for the extraction of readily soluble SO_4^{2-} from organic soils employs a 0.01 M NH_4Cl extraction solution. Sulphate in the extract is then quantified by ion chromatography. This method is primarily for use in the determination of extractable sulphate contents in organic soil horizons.

Summary of Method

Field-moist organic horizons are homogenized in a Waring blender and extracted with 0.01 M NH_4Cl (approx. 2:20 air-dried soil:solution). Sulphate in the extracts is determined by suppressor or single column ion chromatography.

Interferences and Shortcomings

Colloidal material and certain organic compounds may interfere with the SO_4^{2-} peak on the chromatogram. Membrane filtering will help remove colloidal material and extend the life of the guard cartridges.

As with other soluble salts in soils, the amount of SO_4^{2-} extracted varies with the soil:extractant ratio. This ratio should, therefore, remain constant if samples are to be compared.

Safety

Wear protective clothing (laboratory coat and gloves) and safety glasses when preparing reagents. Follow the safety precautions of the manufacturer when operating instruments.

Apparatus and Equipment

- ion chromatograph, pump and conductivity detector. Either suppressed ion chromatography (SIC) such as the Dionex models, or single column ion chromatography (SCIC) such as the Waters systems.
- anion separator column and appropriate guard column.
- automated sampler and injection system and sample vials or tubes to fit sampler.
- data recording system, integrator or strip chart recorder.
- balance, accurate to 0.001 g.
- reciprocal shaker.
- vacuum filtration apparatus and funnels.
- vacuum membrane filter apparatus (optional).
- volumetric flask, 1 L.
- Nalgene bottles, 60 mL.

Reagents and Consumable Materials

- ammonium chloride (NH_4Cl), reagent grade powder.
- sodium sulphate (Na_2SO_4), reagent grade crystals.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- NH_4Cl extracting solution, 0.01 M--Weigh out 0.5350 g NH_4Cl and transfer to a 1 L volumetric flask. Dissolve in DI and dilute to one litre.
- sulphate standard stock solution (1,000 mg/L SO_4)--Weigh 1.4790 g Na_2SO_4 (oven dried at 105°C and cooled in a desiccator) and transfer to a 1 L volumetric flask. Dissolve in NH_4Cl extracting solution and dilute to one litre. Store refrigerated at 4°C .
- working sulphate standards prepared from the stock solution to cover expected range of SO_4^{2-} concentrations in sample extracts. Use NH_4Cl extracting solution during dilution of the standard stock solution.
- appropriate eluent for the IC system and anion separator column used. (see manufacturer's recommendations).
- filter paper, Whatman #42.
- membrane filters, $0.45\ \mu\text{m}$ (optional).

Calibration and Standardization

Follow the set-up procedures outlined in the manufacturer's operating manual for the specific instrument. Pump eluent through the system and stabilize the baseline. Adjust the recorder until zero is approximately 10% and the high standard is approximately 90% of the chart.

Use a minimum of three standards plus a zero standard to calibrate the system. The standards are analyzed and a calibration curve produced by plotting peak area or height against concentration. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded. The calibration curve should be close to linear. Calibration standards should be prepared fresh daily. Calibration standards should be prepared in the extraction solution.

Procedure

- Step 1 - Thaw sample if previously frozen.
- Step 2 - Homogenize field moist sample in a Waring blender until visually homogeneous.
- Step 3 - Weigh an aliquot of the field-moist soil that would approximate 2 g on a dry weight basis into a 60-mL Nalgene bottle.

NOTE: An additional aliquot should be prepared for drying and determination of the % moisture so that SO_4^{2-} can be calculated on a dry weight basis.

- Step 4 - Add 20 mL of 0.01 M NH_4Cl extracting solution to each bottle.
- Step 5 - Shake for 1 h on a reciprocal shaker at 1 to 2 cycles per second.
- Step 6 - Vacuum filter the resulting suspension using Whatman #42 filter paper in a Buchner funnel.

NOTE: Alternately, samples may be centrifuged until the supernatant is clear.

- Step 7 - Vacuum filter the samples through 0.45 μm membrane filters.
- Step 8 - Analyze samples by ion chromatography within 24 hours.

NOTE: Samples may have to be transferred into vials or tubes specific to the type of sampler used.

NOTE: Samples should be stored at 4° C prior to sample analysis.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

The concentration of SO_4^{2-} is calculated by reading the peak area (or height) of the samples against the standard calibration curve. The calculation program used should take into account sensitivity changes throughout the run as detected by the in-run standard. All sample results are method blank subtracted.

For organic samples, the sample weight must be based on % moisture in the field-moist sample. Therefore, the following formula is applicable:

$$\text{Extractable } \text{SO}_4^{2-} \text{ (mg S/kg)} = \frac{\text{SO}_4^{2-} \text{ (mg/L) in solution} \times \text{extract volume (mL)}}{(\text{initial sample weight (g)} \times \text{moisture correction})}$$

NOTE: The moisture correction factor is as follows with the moisture content presented as a fraction of the whole (e.g., 0.75):

$$[(1 - \text{moisture content in \%}) \div (100 + \text{moisture content in \%})]$$

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Water Extractable Sulphate

Introduction

Sulphur, in the form of sulphate (SO_4^{2-}), is the principal anion in acidic deposition in eastern North America. Concern over the long-range transport and deposition of SO_4^{2-} in precipitation has lead to increased monitoring of the sulphur status of soils. In most mineral soils, the majority of extractable S is in the SO_4^{2-} form. In contrast, in organic horizons up to 50% of the total extractable S may be organically-bound (Maynard et al., 1987). The ability of soils to adsorb sulphate is one of the principal factors affecting the rate and extent of soil and watershed response to acidic deposition. Quantification of existing pools of adsorbed sulphate on a soil, concurrent with measurements of sulphate adsorption capacity of that soil, provide useful information for understanding the status and for predicting the future response of the soil to acidic deposition. The measurement of extractable sulphate is, therefore, suggested for terrestrial monitoring programs, such as LRTAP.

Review of Methods

The preparation and storage of soils is important in the determination of extractable SO_4^{2-} in soils. Several studies have shown that drying significantly altered the SO_4^{2-} content of the soil, particularly in organic horizons (Peverill et al., 1975; David et al., 1982; Searle and Sparling, 1987). Moreover, storage of air-dried samples at room temperature (20 to 25° C) for between 12 and 78 weeks resulted in significant increases in SO_4^{2-} concentrations (Maynard et al., 1987; Searle and Sparling, 1987). The increased sulphate concentrations observed were not consistent among soils. For extractable SO_4^{2-} measurements in organic horizons to be meaningful, the test should be performed on field-moist samples. A representative sample can be obtained by mixing/homogenizing the moist organic horizon prior to analysis. The changes in SO_4^{2-} concentration in mineral soils caused by drying and storage were much less than organic soils. For this reason and because of the difficulty in obtaining a representative sample from moist samples, air-dried soils are recommended for the analysis of mineral soils.

Numerous extractants have been proposed for the removal of SO_4^{2-} from soils (Beaton et al., 1968; Tabatabai, 1982). Most of the experimental work has involved using mineral agricultural soils in an attempt to correlate extractable SO_4^{2-} with plant growth and S uptake. Many of the techniques used on agricultural soils have been adapted for use on forest soils.

Sulphate extraction methods in soils can be broken into two groups based on the fraction of SO_4^{2-} removed. The two groups include those extractants that remove readily soluble SO_4^{2-} and those extractants that remove readily soluble SO_4^{2-} plus adsorbed SO_4^{2-} . The extractants used to remove readily soluble SO_4^{2-} (H_2O and weak salt solutions, such as CaCl_2 or NH_4Cl), are preferred for organic and mineral soils containing no appreciable amounts of adsorbed SO_4^{2-} . In contrast,

phosphate extractants, generally employing a $\text{Ca}(\text{H}_2\text{PO}_4)_2$ solution (500 mg P/L), are recommended for soils containing sesquioxides, allophane, or kaolinitic clays which have the potential to adsorb SO_4^{2-} . The latter class of soils generally include the mineral horizons of most forest soils.

Eight extractants (including H_2O , several weak salts, and $\text{Ca}(\text{H}_2\text{PO}_4)_2$) were evaluated for extractable SO_4^{2-} on five forest organic soil horizons (Maynard et al., 1987). The most consistent extractant for SO_4^{2-} was 0.01 M NH_4Cl (1:10 soil to solution ratio), although all the weak salt extractants removed similar amounts of SO_4^{2-} . Water as an extractant gave the most variable results. The phosphate extractant did not remove any more SO_4^{2-} in the organic soils and gave more variable results than the NH_4Cl extractant. The strong phosphate solution was also noted to reduce the life of the ion chromatography column.

A phosphate extractant is preferred for mineral soils to ensure that strongly adsorbed SO_4^{2-} is extracted. Calcium dihydrogen phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) solution, containing 500 mg/L P, is preferred over Na or K phosphates since Ca enhances particle flocculation in clayey soils and makes filtering more convenient.

Over the last decade, the development of ion chromatography (IC) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have made the quantification of S and SO_4^{2-} rapid and more accurate. The determination of total S in a soil extract can be done by ICP-AES, but this technique is not specific for SO_4^{2-} . Ion chromatography is, however, specific to the SO_4^{2-} ion (Dick and Tabatabai, 1979; Nieto and Frankenberger, Jr., 1985; Maynard et al., 1987) and is, therefore, preferred for sulphate quantification in soil extracts.

Reference Method

The reference method for the extraction of readily soluble SO_4^{2-} from mineral soils employs deionized water as the extraction solution. Sulphate in the extract is then quantified by ion chromatography. This method is primarily for use in the determination of readily soluble sulphate contents in mineral soil horizons. However, it may be used in place of the NH_4Cl extraction procedure for organic horizons bearing in mind the method limitations indicated in the "Review of Methods" section.

Summary of Method

Air-dried mineral horizon samples, which have been disaggregated and passed through a 2-mm sieve, are extracted with deionized water (1:20 air-dried soil:water). Sulphate in the extracts is determined by suppressor or single column ion chromatography.

Interferences and Shortcomings

Colloidal material and certain organic compounds may interfere with the SO_4^{2-} peak on the chromatogram. Membrane filtering will help remove colloidal material and extend the life of the guard cartridges.

As with other soluble salts in soils, the amount of SO_4^{2-} extracted varies with the soil:extractant ratio. This ratio should, therefore, remain constant if samples are to be compared.

Safety

Wear protective clothing (laboratory coat and gloves) and safety glasses when preparing reagents. Follow the safety precautions of the manufacturer when operating instruments.

Apparatus and Equipment

- ion chromatograph, pump and conductivity detector. Either suppressed ion chromatography (SIC) such as the Dionex models, or single column ion chromatography (SCIC) such as the Waters systems.
- anion separator column and appropriate guard column.
- automated sampler and injection system and sample vials or tubes to fit sampler.
- data recording system, integrator or strip chart recorder.
- balance, accurate to 0.001 g.
- reciprocal shaker.
- vacuum filtration apparatus and funnels.
- vacuum membrane filter apparatus (optional).
- volumetric flask, 1 L.
- Nalgene bottles, 100 mL.

Reagents and Consumable Materials

- sodium sulphate (Na_2SO_4), reagent grade crystals.
- water--DI water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).
- sulphate standard stock solution (1,000 mg/L SO_4)--Weigh 1.4790 g Na_2SO_4 (oven dried at 105° C and cooled in a desiccator) and transfer to a 1 L volumetric flask. Dissolve in DI water and dilute to one litre. Store refrigerated at 4° C.
- working sulphate standards prepared from the stock solution to cover expected range of SO_4^{2-} concentrations in sample extracts.
- appropriate eluent for the IC system and anion separator column used. (see manufacturer's recommendations).
- filter paper, Whatman #42.
- membrane filters, 0.45 μm (optional).

Calibration and Standardization

Follow the set-up procedures outlined in the manufacturer's operating manual for the specific instrument. Pump eluent through the system and stabilize the baseline. Adjust the recorder until zero is approximately 10% and the high standard is approximately 90% of the chart.

Use a minimum of three standards plus a zero standard to calibrate the system. The standards are analyzed and a calibration curve produced by plotting peak area or height against concentration. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded. The calibration curve should be close to linear. Calibration standards should be prepared fresh daily. Calibration standards should be prepared in the extraction solution, in this case DI water.

Procedure

Step 1 - Weigh 4.00 g of air-dried mineral soil into a 100-mL Nalgene bottle.

NOTE: If this method is to be used for an organic soil horizon, homogenize the sample in a Waring blender until visually homogeneous and weigh a 2.00 gram sample into the 100-mL Nalgene bottle.

NOTE: An additional aliquot should be prepared for drying and determination of the % moisture so that SO_4^{2-} can be calculated on a dry weight basis.

Step 2 - Add 80 mL of DI water to each bottle.

Step 3 - Shake for 1 h on a reciprocal shaker at 1 to 2 cycles per second.

Step 4 - Vacuum filter the resulting suspension using Whatman #42 filter paper in a Buchner funnel.

NOTE: Alternately, samples may be centrifuged until the supernatant is clear.

Step 5 - Vacuum filter the samples through 0.45 μm membrane filters.

Step 6 - Analyze samples by ion chromatography within 24 hours.

NOTE: Samples may have to be transferred into vials or tubes specific to the type of sampler used.

NOTE: Samples should be stored at 4° C prior to sample analysis.

Quality Control

Precision

One sample should be analyzed in duplicate with each run of thirty samples. To eliminate bias due to position in the run, the routine sample duplicate should be analyzed separately within the analytical run. Within-run precision is determined from duplicates based on relative percent difference between the samples at an acceptance limit of a RPD $\leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Three method blanks, carried through the extraction procedure, are analyzed with each batch of samples for each cation to measure potential contamination. Method blanks should be run at the beginning, middle, and end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Quality Control Check Standard

A quality control check standard (QCCS) should be analyzed at the beginning, after every ten samples, and after the last sample of each analytical run. The QCCS should contain all the analytes of interest with mid-calibration range concentrations. Quantified values of the QCCS should be within $\pm 10\%$ of the known concentration of the standard.

It is highly recommended that the concentrations of this sample be consistent through time so that control charts may be plotted to monitor laboratory bias and other potential problems. If analyzed values deviate from the long-term mean by more than three standard deviations, the instrument is re-standardized and re-calibrated prior to any further sample analyses.

Suggested Run Format

QCCS, MB, Samples 1 to 8, QCPS,
QCCS, Samples 9 to 16, MB, DUP,
QCCS, Samples 16 to 25, SRM,
QCCS, Samples 26 to 30, MB, QCCS.

where: QCCS = quality control check standard
MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

The concentration of SO_4^{2-} is calculated by reading the peak area (or height) of the samples against the standard calibration curve. The calculation program used should take into account sensitivity changes throughout the run as detected by the in-run standard. All sample results are method blank subtracted.

For mineral soils, calculate extractable sulphate concentrations as follows:

$$\text{Extractable } \text{SO}_4^{2-} \text{ (mg S/kg)} = \frac{\text{SO}_4^{2-} \text{ (mg/L) in solution} \times \text{extract volume (mL)}}{\text{dry soil weight (g)}}$$

For organic samples, the sample weight must be based on % moisture in the field-moist sample. Therefore, the following formula is applicable:

$$\text{Extractable } \text{SO}_4^{2-} \text{ (mg S/kg)} = \frac{\text{SO}_4^{2-} \text{ (mg/L) in solution} \times \text{extract volume (mL)}}{(\text{initial sample weight (g)} \times \text{moisture correction})}$$

NOTE: The moisture correction factor is as follows with the moisture content presented as a fraction of the whole (e.g., 0.75):

$$[(1 - \text{moisture content in \%}) \div (100 + \text{moisture content in \%})]$$

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Total Sulphur

Introduction

Sulphur is an essential element for all biological systems. Historically, its importance as a plant nutrient and reports of widespread deficiencies, especially in sub-humid areas of intensive leaching, has made it a routine measurement in some laboratories. Most of the sulphur in surface soils occurs in combination with organic matter (Freney and Williams, 1983). Inorganic SO_4^{2-} -S, although it comprises a small fraction of the total S in most soils, is important to the cycling of S in the soil.

Concern over the long-range transport and deposition of SO_4^{2-} in precipitation has led to increased monitoring of the sulphur status of soils. The determination of total sulphur is useful for characterizing relationships between inputs of sulphur from acidic deposition and soil sulphur pools. Fundamental to studying the effects of strong acid precipitation on terrestrial and aquatic systems is an understanding of the behaviour of sulphur in these systems. The measurement of total S is, therefore, suggested for terrestrial monitoring programs.

Review of Methods

It has only been within the last 15 years that the difficulty of accurately measuring S in soils has been overcome. Recent advances in analytical techniques have resulted in the accurate and precise measurement of S in various types of soils (Dick and Tabatabai, 1979; Hogan and Maynard, 1984; Nieto and Frankenberger, Jr., 1985; Maynard et al., 1987). Several methods are routinely used for the determination of S in environmental samples. These may be divided into two groups, namely, those involving wet oxidation of the sample and those which involve direct sample analysis (Hogan and Maynard, 1984).

Methods available for the wet oxidation of organic materials are well documented (Beaton et al., 1968; Tabatabai, 1982; Blanchar, 1986). Acid and alkaline oxidation are the most common (Blanchar et al., 1965; Tabatabai and Bremner, 1970), as they are dependable, accurate and relatively rapid (Blanchar, 1986). Full recovery from an acid digestion usually requires the use of perchloric acid. The danger associated with its use and the special facilities required have meant that, until recently, acid digestions have been avoided. The recent adaptation of microwave ovens for use in the laboratory has led to the development of microwave acid oxidation digestion techniques for foliage and soils which successfully use hydrogen peroxide in place of perchloric acid. Acid digestions for the measurement of total S in soils requires the use of hydrofluoric acid to destroy the silicate matrix and ensure complete recovery of S. A HNO_3 - HClO_4 -HF mixture is commonly used. For this reason, many analysts have preferred the safer, more rapid, dry combustion techniques.

The wet oxidation technique converts S to SO_4^{2-} and produces a solution which can be analyzed by a variety of methods. Turbidimetry is insensitive, lacks precision and is subject to numerous interferences (Beaton et al., 1968). Colourimetric methods such as the methylene blue technique (Technicon, 1972) also have limited application in soils and plant analysis because of interferences by major nutrient cations (Maynard et al., 1987). The colourimetric method developed by Johnson and Nishita (1952) was found to be the most sensitive and accurate of the colourimetric procedures. Ion chromatography has not been used extensively for the measurement of total S, but has been shown to be an excellent method for the determination of SO_4^{2-} and its companion anions, as well as for cation determinations, in waters and soil extracts.

Recent publications (Hogan and Maynard, 1984; White and Douthit, 1985; Novozamsky et al., 1986) have shown that the accurate and precise measurement of S is possible by ICP-AES in a range of environmental samples. Precision is estimated at $\pm 2\%$. Results of analysis of NIST plant material and sediments demonstrated that the precision and accuracy obtained by ICP-AES are equal to or better than any other technique currently used. The ICP-AES has several advantages over other methods. It is rapid, flexible, has a dynamic range, is free from interferences and permits simultaneous multielement analysis. These factors make it a preferred method of analysis for laboratories possessing ICP-AES capabilities.

Direct analysis of the sample may be done by combustion of the sample at elevated temperatures (generally at 1000°C or higher) and measuring the liberated SO_2 by an infrared detector. Examples of instruments that combust the sample include the LECO combustion furnace, Carla-Erba combustion furnace, or the Fisher S analyzer. These methods require little or no sample preparation for the determination of total S.

Additionally, X-ray fluorescence (XRF) may be used to quantify soil S along with many other elements. The method has been used to a lesser extent on soils than the aforementioned methods (Tabatabai and Bremner, 1975). The use of XRF technology is, however, a tedious, time consuming process. Sample preparation may include sample fusion into a borate glass disk or pressing the sample into a pellet under high pressure. Unfortunately, without fusion of the sample into a glass, the natural variability in the sample matrix causes difficulty in obtaining representative standards and can result in interelement interferences. Finally, the expense of the instrumentation makes this method an unfeasible option for many laboratories.

The LECO S analyzer was originally developed for the determination of S in steel, but because of its simplicity, speed, and convenience, it has been adapted for use in soil and plant analysis (Tabatabai, 1982). An initial evaluation of this method by Tabatabai and Bremner (1970) showed total S results to be unsatisfactory for research that required accurate and precise determinations. The Ontario Ministry of the Environment has found that the infrared detection system on the LECO gave unacceptable results for highly organic soil samples.

Reference Method

The reference method for total S in soil samples is dry combustion in a LECO sulphur analyzer with infrared detection. The analysis of S by LECO-S analyzer has been chosen as a reference method because it is widely used in North America and has been used successfully by some laboratories for both soil and foliage samples. However, the use of other manufacturer's instruments for total sulphur determinations is acceptable.

Summary of Method

An air-dried and finely ground soil sample is heated with an accelerator to 1600°C in a stream of high purity oxygen. The released sulphur is converted to sulphur dioxide and is detected by infrared detector (Hern, 1984).

Interferences and Shortcomings

Large amounts of carbon can prevent proper ignition of the sample. With incomplete combustion, a poor recovery of S may be obtained. This may be overcome by reducing the sample size and adding LECO Iron Chip Accelerator plus tin. Preashing of highly organic soils at 475 to 500°C for two hours may be performed, but preliminary studies should be done on the type of

samples to be analyzed to determine whether a loss of S occurs during this ashing. Preashed samples also require accelerator to overcome interference from residual carbon.

Moisture deposits on the walls of the delivery tubes or the surface of the dust filter will absorb SO_2 . This may be overcome by using magnesium perchlorate between the dust filter and the original drying tube.

Interferences from nitrogen may be overcome by increasing the oxygen flow rate to 1.5 litres/minute.

Safety

Normal safety precautions should be taken when using high-temperature combustion furnaces.

Protective clothing and safety glasses should be worn when handling reagents. Heat resistant gloves may be needed when placing samples in the furnace. The furnace must be adequately vented and protected from human contact and combustible materials. Gas cylinders should be bolted or chained in an upright position.

Fumes of magnesium oxide are toxic. Magnesium perchlorate is a fire and explosion hazard if it comes in contact with organic materials.

Apparatus and Equipment

- Sulphur analyzer with infrared detector, LECO model SC-132, or equivalent.
- Balance, accurate to 0.001 g.
- LECO scoop.

Reagents and Consumable Materials

- Oxygen, high purity.
- Compressed air, if needed.
- Anhydrous magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$), 10-20 mesh, or equivalent desiccant specified by manufacturer for drying gases after combustion and prior to detection.
- Magnesium oxide, MgO , reagent grade powder, low in sulphur.
- LECO combustion boats, sulphur-free and appropriate for use with the equipment used.
- Accelerators, vanadium pentoxide, iron chips, and/or copper metal

Calibration and Standardization

Set up the instrument according to the LECO operating manual. In general, the instrument should be calibrated at least once a day or once per batch of samples, whichever is more frequent.

Use either NIST (formerly NBS) reference materials or standards supplied by the manufacturer and approved by the laboratory or QA manager. The concentration range of the standards must be representative of the C concentrations expected in the soil samples. A minimum of a two-point calibration curve should be used. Use of a NIST standard reference material as an initial calibration check is highly recommended.

Some suggested calibration standards and reference samples include: LECO brand iron powder (0.036% S), LECO coal calibration standard (2.56% S), NIST (NBS) coal reference material (1.89% S), SU-1A nickel-copper-cobalt ore (9.35% S), and CCU-1 reference material (35.4% S).

Ensure that the anhydrous is dry and the dust filter is clean. Prior to analyzing samples, the instrument is conditioned by running low level calibration standards until the results are stabilized to within 5%. Once stable, three blank crucibles (accelerator only) are analyzed followed by three standards.

Procedure - Infra-red Detection

- Step 1 - Weigh out 0.250 g air dried and finely ground soil into a LECO combustion boat. Record sample weight.
- Step 2 - Preset power settings on induction furnace according to the manual.
- Step 3 - Ignite the sample in the crucible for the suggested time period. Seven minutes has been found to work well for soils but this should be determined for the range of soil types specific to the laboratory.
- Step 4 - Take a sulphur measurement reading.

Quality Control

Precision

One sample from each batch should be analyzed in duplicate. Within-run precision is determined from duplicates based on relative percent difference (RPD) between the samples with an acceptance limit of a $RPD \leq 10\%$.

Accuracy

Accuracy is determined by analysis of a standard reference material (SRM). Acceptable limits for accuracy should be $\pm 10\%$ from the known concentration of the standard or within the accuracy windows supplied by the reference material manufacturer, whichever is larger.

Method Blanks

Two blank crucibles (accelerator only) are analyzed before the run and one blank crucible is also analyzed in the middle and at the end of each analytical run. The concentration of each blank should be less than or equal to the instrument detection limit. All results should be blank corrected using the mean of the acceptable method blank readings.

Quality Control Preparation Sample

A matrix matched in-house quality control preparation sample (QCPS) should be analyzed once per analytical run. This sample is used to monitor accuracy and long-term between-run precision. Accuracy of the QCPS should be within $\pm 10\%$ of the long-term mean. Between-run precision can be determined by analyzing the QCPS and calculating the cumulative long-term standard deviation. If values plotted on a control chart deviate from the long-term mean by more than three standard deviations, the run should be completely reanalyzed, including all digestion and quantification steps.

Suggested Run Format

MB, MB, Samples 1 to 8, QCPS,
Samples 9 to 16, MB, DUP,
Samples 16 to 25, SRM,
Samples 26 to 30, MB.

where: MB = method blank
QCPS = quality control preparation sample
DUP = duplicate sample
SRM = standard reference material

Calculations and Reporting

Infra-red system: % S is read directly from the instrument. The sample weight, as different from the standard, is taken into account on some instruments.

Results are reported to two significant figures. Results are read to the nearest 0.001%. Results should be blank corrected.

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