Soil Sampling and Methods of Analysis

Second Edition

In physical science the first essential step in the direction of learning any subject is to find principles of numerical reckoning and practicable methods for measuring some quality connected with it. I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the state of science, whatever the matter may be.

Lord Kelvin, Popular Lectures and Addresses (1891–1894),
vol. 1, *Electrical Units of Measurement*

Felix qui potuit rerum cognoscere causas.

Happy the man who has been able to learn the causes of things.

Virgil: Georgics (II, 490)

Soil Sampling and Methods of Analysis

Second Edition

Edited by
M.R. Carter
E.G. Gregorich

Canadian Society of Soil Science

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PREFACE

This volume is an update of the book, *Soil Sampling and Methods of Analysis*, first published in 1993. The aims of this second edition remain the same as those of the earlier edition—to provide a compilation of soil analytical and sampling methods that are commonly used, straightforward, and relatively easy to use. The materials and procedures for these methods are presented with sufficient detail and information, along with key references, to characterize the potential and limitation of each method.

As methods develop, so do their degree of sophistication. Taking these developments into account, the second edition includes several chapters that serve as ''primers,'' the purpose of which is to describe the overall principles and concepts behind a particular type or types of measurement, rather than just methods alone.

All of the chapters retained from the earlier edition have been modified and updated. The second edition also introduces new chapters, particularly in the areas of biological and physical analyses, and soil sampling and handling. For example, the ''Soil Biological Analyses'' section contains new chapters to reflect the growing number and assortment of new microbiological techniques and the burgeoning interest in soil ecology. New chapters are offered describing tools that characterize the dynamics and chemistry of soil organic matter. A new section devoted to soil water presents up-to-date field- and laboratory-based methods that characterize saturated and unsaturated soil hydraulic properties.

This second edition of *Soil Sampling and Methods of Analysis* comprises 7 sections and a total of 85 chapters and 2 appendices written by 140 authors and co-authors. Each section is assembled by two section editors and each chapter reviewed by at least two external reviewers. We are grateful to these people for their diligent work in polishing and refining the text and helping to bring this new volume to fruition. We particularly thank Elaine Nobbs for her support in working with the many authors involved in writing this book.

We offer this new edition of *Soil Sampling and Methods of Analysis* in the belief that it will continue as a useful tool for researchers and practitioners working with soil.

M.R. Carter and E.G. Gregorich
Editors

CANADIAN SOCIETY OF SOIL SCIENCE

The Canadian Society of Soil Science is a nongovernmental, nonprofit organization for scientists, engineers, technologists, administrators, students, and others interested in soil science. Its three main objectives are

- To promote the wise use of soil for the benefit of society
- To facilitate the exchange of information and technology among people and organizations involved in soil science
- To promote research and practical application of findings in soil science

The society produces the international scientific publication, the *Canadian Journal of Soil Science*, and each year hosts an international soil science conference. It sponsored the first edition of *Soil Sampling and Methods of Analysis* (Lewis Publishers, CRC Press, 1993) and also promoted the publication of the popular reference book *Soil and Environmental Science Dictionary* (CRC Press, 2001). The society publishes a newsletter to share information and ideas, and maintains active liaison and partnerships with other soil science societies.

For more information about the Canadian Society of Soil Science, please visit www.csss.ca.

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M.R. Carter holds degrees in agriculture and soil science from the University of Alberta and obtained a PhD in soil science from the University of Saskatchewan in 1983. Since 1977, he has held agricultural research positions with Agriculture and Agri-Food Canada (AAFC) and is currently a research scientist at the AAFC Research Center, Charlottetown, Prince Edward Island. Dr. Carter is a fellow and past-president of the Canadian Society of Soil Science, and past editor of the *Canadian Journal of Soil Science*. He edited the first edition of *Soil Sampling and Methods of Analysis*, (CRC Press, 1993) and also edited *Conservation Tillage in Temperate Agroecosystems* (CRC Press, 1994) and *Structure and Organic Matter Storage in Agricultural Soils* (CRC Press, 1996). In collaboration with Dr. Gregorich, he edited *Soil Quality for Crop Production and Ecosystem Health* (Elsevier, 1997) and *Soil & Environmental Science Dictionary* (CRC Press, 2001). Dr. Carter presently serves as editor-in-chief for the international scientific journal *Agriculture Ecosystems & Environment*.

E.G. Gregorich is a research scientist with Agriculture and Agri-Food Canada at the Central Experimental Farm in Ottawa, Canada. His work focuses on soil biochemistry, particularly carbon and nitrogen cycling in soil. He is a fellow and past-president of the Canadian Society of Soil Science, and has served the Soil Science Society of America as chair of the soil biology and biochemistry division. Dr. Gregorich has been a member of the International Panel on Climate Change, has conducted field studies in Scotland, New Zealand, and Antarctica, and directs a Canadian international development project in Vietnam. He has served as associate editor for the *Journal of Environmental Quality*; *Agriculture, Ecosystems & Environment*; *European Journal of Soil Science*; and the *Canadian Journal of Soil Science*. This is the third book on which he and Dr. Carter have collaborated as editors.

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Chapter 1
Soil Sampling Designs

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1.1 INTRODUCTION

Sampling involves the selection from the total population of a subset of individuals upon which measurements will be made; the measurements made on this subset (or sample) will then be used to estimate the properties (or parameters) of the total population. Sampling is inherent to any field research program in soil science because the measurement of the total population is impossible for any realistic study. For example, even a single 10 ha field contains about 100,000 1 m^2 soil pits or 1×10^7 10 cm^2 cores, and sampling of the entire population would be more of an unnatural obsession than a scientific objective.

Sampling design involves the selection of the most efficient method for choosing the samples that will be used to estimate the properties of the population. The definition of the population to be sampled is central to the initial formulation of the research study (Eberhardt and Thomas 1991; Pennock 2004). The sampling design defines how specific elements will be selected from the population, and these sampled elements form the sample population.

There are many highly detailed guides to specific sampling designs and the statistical approaches appropriate for each design. The goal of this chapter is to present the issues that should be considered when selecting an appropriate sampling design. In the final section, specific design issues associated with particular research designs are covered. Suggested readings are given in each section for more in-depth study on each topic.

1.2 APPROACHES TO SAMPLING

1.2.1 Haphazard, Judgment, and Probability Sampling

Sample locations can be chosen using (a) haphazard sampling, (b) judgment sampling, or (c) probability sampling. Haphazard, accessibility, or convenience sampling involves a series of nonreproducible, idiosyncratic decisions by the sampler and no systematic attempt is made to ensure that samples taken are representative of the population being sampled. This type of sampling is antithetical to scientific sampling designs. Judgment sampling (also termed purposive sampling [e.g., de Gruijter 2002]) involves the selection of sampling points based on knowledge held by the researcher. Judgment sampling can result in accurate estimates of population parameters such as means and totals but cannot provide a measure of the accuracy of these estimates (Gilbert 1987). Moreover the reliability of the estimate is only as good as the judgment of the researcher. Probability sampling selects sampling points at random locations using a range of specific sample layouts, and the probability of sample point selection can be calculated for each design. This allows an estimate to be made of the accuracy of the parameter estimates, unlike judgment sampling. This allows a range of statistical analyses based on the estimates of variability about the mean to be used, and is by far the most common type of sampling in soil science.

1.2.2 Research Designs Using Judgment Sampling

Pedogenetic and soil geomorphic studies focus on determining the processes that formed the soil properties or landscapes under study and the environments that controlled the rates of these processes. Pedon-scale studies are closely associated with the development of soil taxonomic systems, and focus on vertical, intrapedon processes. Soil geomorphic studies are the interface between quaternary geology and soil science, and soil geomorphologists focus on lateral transfer processes and the historical landscape evolution.

Both types of studies involve the identification of soil and/or sediment exposures that are highly resolved records of the sequence of processes that have formed the soil landscape. The researcher locates these exposures by using his judgment as to the landscape positions where optimum preservation of the soil–sediment columns is most likely. The development of the chronological sequence can be done with a detailed analysis of a single exposure; no replication of exposures is required.

Surveys are designed to define the extent of spatial units. Soil surveyors map the distribution of soil taxonomic units and provide descriptive summaries of the main properties of the soils. In soil survey the association between soil classes and landscape units is established in the field by judicious selection of sampling points (termed the free survey approach). This type of judgment sampling can be an extremely efficient way of completing the inventory. Contaminant surveys are most typically undertaken by private-sector environmental consultants, and the specific objective may range from an initial evaluation of the extent of contamination to the final stage of remediation of the problem. Laslett (1997) states that consultants who undertake these surveys almost always employ judgment sampling and place their samples where their experience and prior knowledge of site history suggest the contamination might be located. In many jurisdictions the sampling design may also be constrained by the appropriate regulatory framework.

1.2.3 Research Designs Using Probability Sampling

Inventory studies share the common goal of measuring the amount of a property or properties under study and the uncertainty surrounding our estimate of the amount. For example, in agronomic sampling we may wish to estimate the amount of plant-available nutrients in a given field; in contaminant sampling the goal may be to estimate the amount of a contaminant present at a site. In comparative mensurative experiments, comparisons are drawn among classes that the researcher defines but cannot control—for example, sampling points grouped by different soil textures, landform positions, soil taxonomic classes, and drainage class. Their location cannot be randomized by the researcher, unlike imposed treatments such as tillage type or fertilizer rates where randomization is essential. In manipulative experiments the treatments can be directly imposed by the researcher—ideally as fixed amounts that are applied precisely. Many studies are hybrid mensurative–manipulative designs—for example, the measurement of yield response to different fertilizer rates (imposed treatment) in different landform positions (characteristic or inherent treatment). The role of sampling in inventory, mensurative, and manipulative designs is very similar—to allow statistical estimation of the distribution of the parent population or populations. In inventory studies the statistical estimates may be the end point of the study.

Pattern studies are undertaken to assess and explain the spatial or temporal pattern of properties. Two main types of pattern studies exist: (a) the quantification of the spatial and temporal variability in properties and (b) hypothesis generation and testing using point patterns. In pattern studies the initial goal may be a visual assessment of the pattern of observations in time or space, and statistical estimation of the populations may be a secondary goal.

Geostatistical and other spatial statistical studies are undertaken to model the spatial pattern of soil properties, to use these models in the interpolation of values at unsampled locations, to assess the suitability of different spatial process models, or to assist in the design of efficient sampling programs.

1.3 STATISTICAL CONCEPTS FOR SAMPLING DESIGN

1.3.1 Measures of Central Tendency and Dispersion

The key characteristics of the distribution of attributes are measures of its central tendency and the dispersion of values around the measure of central tendency. In the initial stage of study formulation the researcher defines the population, which is composed of the sampling units and one or more attributes measured on these sampling units. Each attribute has a distribution of values associated with it, which can be characterized by parameters such as the population mean (μ) and variance (σ^2). A sample of the sampling units is drawn from the population, and statistics such as the sample mean ($\bar{x}$) and variance (s^2) are calculated, which serve as estimates of the population parameters. Calculations of these statistics are readily available and will not be repeated here. The number of samples taken is denoted as n. For sample populations that are more or less normally distributed the arithmetic mean ($\bar{x}$) is an appropriate measure of central tendency. The variance (s^2) is a common measure of the deviation of individual values from the mean and its square root; the standard deviation (s)

reports values in the same units as the mean. The coefficient of variation (CV) is a normalized measure of the amount of dispersion around the mean, and is calculated by

$$\mathrm{CV} = (s/\bar{x})100 \tag{1.1}$$

Sample populations in the soil science commonly show a long tail of values to the right of the distribution (i.e., they are right-skewed). In this case a log normal or other right-skewed distribution should be used.

The mathematical properties of the normal distribution are well understood and the probability that the true population mean lies within a certain distance of the sample mean can be readily calculated. For sample populations the estimated standard error of the sample mean is

$$s(\bar{x}) = s/\sqrt{n} \tag{1.2}$$

For a sample population that has a large sample size or where the standard error is known and that approximates a normal distribution, the true mean will be within ± 1.96 standard errors of the sample mean 95 times out of 100 (i.e., where the probability $P = 0.05$). The range defined these limits are the 95% confidence interval for the mean and these limits are the 95% confidence limits. The value 1.96 is derived from the t distribution, and values of t can be derived for any confidence limit. For sample populations based on a small sample size or where the standard error is not known the value of 1.96 must be replaced by a larger t-value with the appropriate degrees of freedom. A probability of exceeding a given standard error (α) may be selected for any sample distribution that approximates the normal distribution and the appropriate confidence limits calculated for that distribution.

1.3.2 Independence, Randomization, and Replication

The goal of sampling is to produce a sample that is representative of the target population. If the choice of samples is not probability based then a strong likelihood exists that the sample will not be representative of the population. For example, selection of sampling locations convenient to a farmyard (instead of distributed throughout the field) may lead to overestimates of soil nutrients due to overapplication of farmyard manure near the source of the manure through time. The use of probability-based sampling designs (i.e., the designs discussed in Section 1.4) confers a design-specific independence on the sample selection process, which satisfies the need for independence of samples required by classical statistical analysis (a theme developed in great detail by Brus and de Gruijter 1997).

Replication is an important consideration in mensurative and manipulative experiments. In a manipulative study, replication is the repeated imposition of a set of treatments (e.g., fertilizer or pesticide rates). In a pattern or mensurative study, replication is the repeated, unbiased selection and sampling of population elements in a particular class—for example, the selection of multiple 5×5 m slope elements in a field that have markedly convex downslope curvatures. Replication provides an estimate of the experimental error, and increasing replication improves precision by reducing the standard error of treatment or class means (Steel and Torrie 1980). Correct identification and sampling of replicates is critical for estimating the parameters of the class the sample is drawn from and is required for statistically correct procedures. Pseudoreplication (Hurlbert 1984) occurs when a researcher assumes a very general effect from a limited sampling and often occurs because the target population has not been clearly defined at the outset of the research.

Randomization is a consideration in manipulative designs. Steel and Torrie (1980, p. 135) summarizes the need for randomization:

> "... it is necessary to have some way of ensuring that a particular treatment will not be consistently favored or handicapped in successive replications by some extraneous sources of variation, known or unknown. In other words, every treatment should have an equal chance of being assigned to any experimental unit, be it unfavorable or favorable."

Randomization is implemented by ensuring the random placement of treatment plots within a field design; the repeated imposition of the same sequence of treatments in a block of treatments may cause an erroneous estimate of the experimental error. The random order of treatment placement is achieved using random number tables or computer-generated randomizations.

1.4 SAMPLE LAYOUT AND SPACING

Although many types of sampling designs exist (reviewed in Gilbert 1987; Mulla and McBratney 2000; de Gruijter 2002) only two main types (random and systematic) are commonly used in the soil and earth sciences. Inventory studies can be completed using any of the designs discussed in the following two sections. Pattern and geostatistical studies typically use transect or grid designs, as is discussed in more detail in Section 1.5.

1.4.1 Simple Random and Stratified Random Sampling

In simple random sampling all samples of the specified size are equally likely to be the one chosen for sampling. In stratified random sampling, points are assigned to predefined groups or strata and a simple random sample chosen from each stratum. The probability of being selected can be weighted proportionally to the stratum size or the fraction of points sampled can vary from class to class in disproportionate sampling. Disproportionate sampling would be used if the degree of variability is believed to vary greatly between classes, in which case a higher number of samples should be drawn from the highly variable classes to ensure the same degree of accuracy in the statistical estimates.

Stratified sampling (correctly applied) is likely to give a better result than simple random sampling, but four main requirements should be met before it is chosen (Williams 1984):

1. Population must be stratified in advance of the sampling.
2. Classes must be exhaustive and mutually exclusive (i.e., all elements of the population must fall into exactly one class).
3. Classes must differ in the attribute or property under study; otherwise there is no gain in precision over simple random sampling.
4. Selection of items to represent each class (i.e., the sample drawn from each class) must be random.

The selection of random points in a study area has been greatly facilitated by the widespread use of Global Positioning System (GPS) receivers in field research. The points to be sampled can be randomly selected before going to the field, downloaded into the GPS unit, and then the researcher can use the GPS to guide them to that location in the field.

TABLE 1.1 Sample Sizes Required for Estimating the True Mean μ Using a Prespecified Relative Error and the Coefficient of Variation

Confidence level	Relative error, d_r	Coefficient of variation (CV), %					
		10	20	40	50	100	150
0.80	0.10	2	7	27	42	165	370
	0.25			6	7	27	60
	0.50				2	7	15
	1.0					2	4
0.90	0.10	2	12	45	70	271	609
	0.25			9	12	45	92
	0.50				2	13	26
	1.0					2	8
0.95	0.10	4	17	63	97	385	865
	0.25			12	17	62	139
	0.50				4	16	35
	1.0					9	16

Source: Adapted from Gilbert, R.O., in *Statistical Methods for Environmental Pollution Monitoring*, Van Nostrand, Reinhold, New York, 1987, 320 pp.

Determination of Sample Numbers in Inventory Studies

A necessary and important step in the planning stages of a project is to determine the number of samples required to achieve some prespecified accuracy for the estimated mean. One approach is to use prior knowledge about the CV of the property under study to estimate sample numbers required to achieve a certain prespecified relative error. The relative error (d_r) is defined as

$$d_r = |\text{sample mean} - \text{population mean}|/\text{population mean} \tag{1.3}$$

The sample numbers required to achieve a specified relative error at a selected confidence level can be estimated from Table 1.1. For example, at a confidence level of 0.95 and a relative error of 0.25, 16 samples are required if the CV is 50% and 139 samples are required if the CV is 150%. Estimates of CV for different soil properties are widely available, and are summarized in Table 1.2.

1.4.2 Systematic Sampling

The most commonly used sampling design for many field studies is systematic sampling using either transects or grids. Systematic sampling designs are often criticized by statisticians but the ease with which they can be used and the efficiency with which they gather information makes them popular in the field of earth sciences. Ideally the initial point of the transect or grid and/or its orientation should be randomly selected. The major caution in the use of systematic sampling with a constant spacing is that the objects to be sampled must not be arranged in an orderly manner which might correspond to the spacing along the transect or the grid.

The choice of a transect or a grid depends on several factors. Certain types of research designs require particular types of systematic designs—as discussed below, wavelet analysis requires long transects whereas geostatistical designs more typically use grid designs. Grids are often used for spatial pattern studies because of the ease with which pattern maps can be derived from the grids. The complexity of landforms at the site is also a consideration.

TABLE 1.2 Variability of Soil Properties

Coefficient of variation			
Low (CV <15%)	**Moderate (CV 15%–35%)**	**High (CV 35%–75%)**	**Very high (CV 75%–150%)**
Soil hue and value[a]	Sand content[a]	Solum thickness[a]	Nitrous oxide flux[b]
pH[a]	Clay content[a]	Exchangeable Ca, Mg, K[a]	Electrical conductivity[b]
A horizon	CEC[a]	Soil nitrate N[b]	Saturated hydraulic conductivity[b]
Thickness[a]	% BS[a]	Soil-available P[b]	Solute dispersion coefficient[b]
Silt content[a]	$CaCO_3$ equivalent[a]	Soil-available K[b]	
Porosity[b]	Crop yield[b]		
Bulk density[b]	Soil organic C[b]		

[a] Adapted from Wilding, L.P. and Drees, L.R., in L.P. Wilding, N.E. Smeck, and G.F. Hall, (Eds.), *Pedogenesis and Soil Taxonomy. I. Concepts and Interactions*, Elsevier Science Publishing, New York, 1983, 83–116.

[b] Adapted from Mulla, D.J. and McBratney, A.B., in M.E. Sumner (Ed.), *Handbook of Soil Science*, CRC Press, Boca Raton, Florida, 2000, A321–A352.

For level and near-level landscapes either a transect or a grid can be used (Figure 1.1). The appropriateness of transects in sloping terrain depends in part on the plan (across-slope) curvature. Where no significant across-slope curvature exists each point in the landscape receives flow from only those points immediately upslope and a single transect can adequately capture the variations with slope position (Figure 1.2). A single transect will not, however, be sufficient if significant plan curvature exists. In this case a zigzag design or multiple, randomly oriented transects could be used, but more typically a grid design is used (Figure 1.3). It is important to ensure that all slope elements are represented in the grid

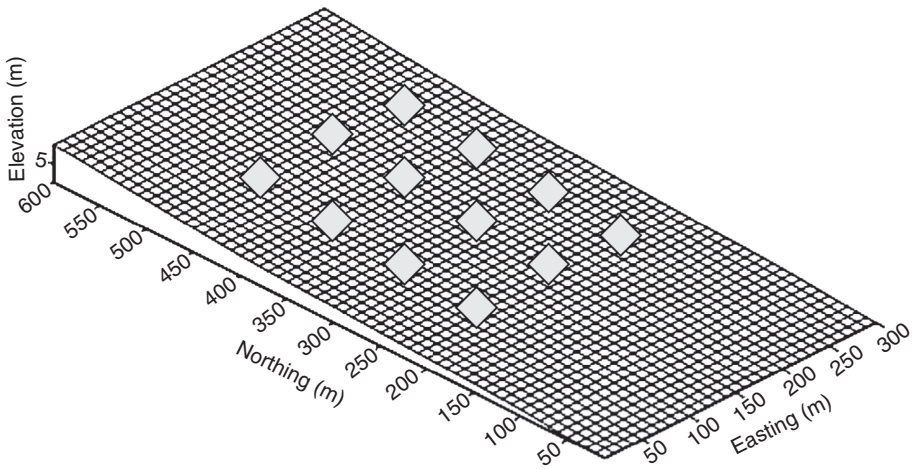

FIGURE 1.1. Example of a grid sampling layout composed of four parallel transects on a near-level surface form. Soil samples would be taken at each point labeled with a diamond shape.

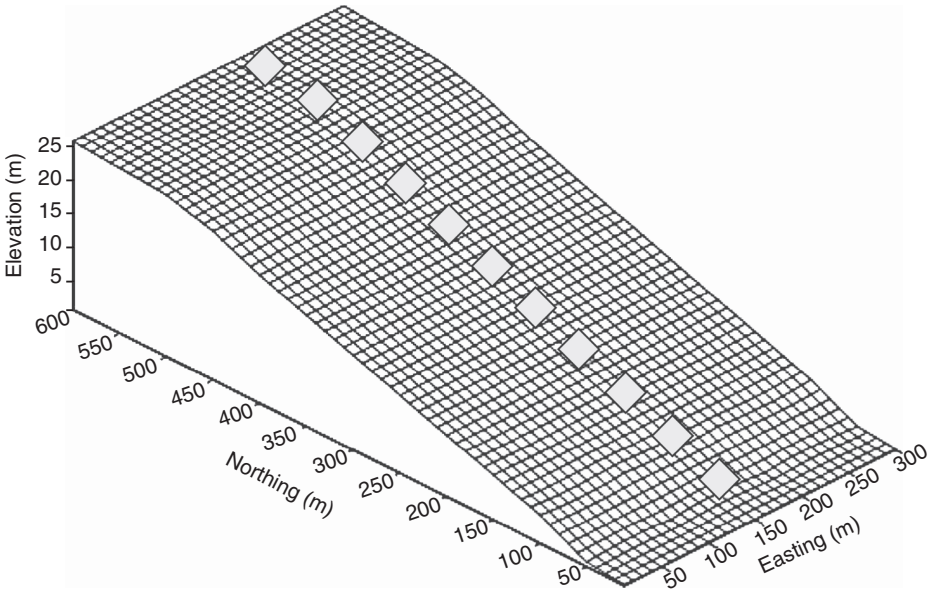

FIGURE 1.2. Example of a transect sampling layout on a sloping surface with no significant across-slope (plan) curvature. Soil samples would be taken at each point labeled with a diamond shape.

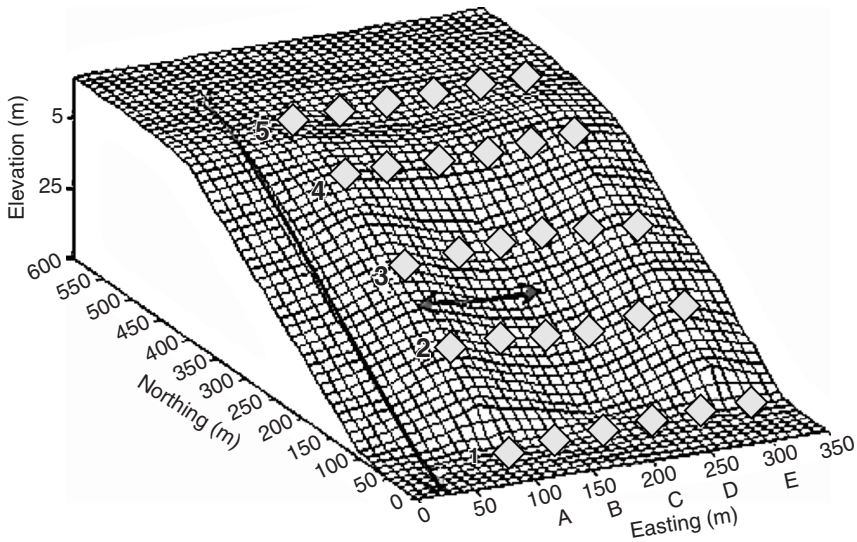

FIGURE 1.3. Example of a grid sampling layout composed of six parallel transects on a sloping surface form with pronounced across-slope curvature. The arrow-oriented downslope delineates the minimum downslope length of the long axis of the grid, and the arrow across the slope indicates the minimum length of the short axis of the grid. Soil samples would be taken at each point labeled with a diamond shape.

design. A rule of thumb is that the grid should extend from the level summit of the slope to the toeslope along the long axis of the slope and along at least one complete convergent–divergent sequence across the slope.

The distance between sampling points in either a transect or a grid should be smaller than the distance required to represent the variability in the field. For example, if the study area contains landforms whose tops and bottoms are equally spaced at 30 m, then a transect crossing these landforms should have sample locations spaced much shorter than this (e.g., 5 or 10 m). It is desirable to base sample spacing on prior knowledge of the area.

1.5 SAMPLING DESIGNS FOR SPECIFIC RESEARCH OBJECTIVES

1.5.1 Sampling Designs for Mensurative and Manipulative Experiments

In mensurative and manipulative designs a typical goal is to assess if the attributes sampled from different classes have different distributions or the same distribution, using difference testing. In the simplest type of hypothesis testing, two hypotheses are constructed: the null hypothesis (H_0) of no difference between the two groups and the alternative hypothesis of a significant difference occurring. The researcher chooses an α level to control the probability of rejecting the null hypothesis when it is actually true (i.e., of finding a difference between the two groups when none, in fact, existed in nature or a Type I error). Peterman (1990) states that the consequences of committing a Type II error (i.e., of failing to reject the null hypothesis when it is, in fact, false) may be graver than a Type I error, especially in environmental sampling. The probability of failing to reject the H_0 when it is, in fact, false is designated as β and the power of a test equals $(1-\beta)$. Calculation of power should be done during the design stage of a mensurative or manipulative experiment to ensure that sufficient samples are taken for a strong test of differences between the groups.

The use of nonstratified, systematic designs may be very inefficient for mensurative experiments. For example, in a landscape where 60% of the site is classified as one class of landform element and 5% is classified into a second class, a 100-point grid should yield approximately 60 points in the major element and 5 points in the second. The dominant element is probably greatly oversampled and the minor element undersampled. Appropriate sample numbers can be efficiently gathered by stratified sampling by a priori placement of points into the relevant groups or strata, and then a random selection of points is chosen within each stratum until the desired number is reached.

In manipulative designs the treatments are commonly applied in small strips (or plots). If the experimental unit is believed to be homogenous then the treatments can be randomly assigned to plots in a completely random design. More typically some degree of heterogeneity is believed to occur—for example, a slight slope or a gradient in soil texture exists across the plot. In this case the treatments are assigned to square or rectangular blocks. Each block typically contains one of each of the treatments being compared in the experiment, and the sequence of treatments in each block is randomly determined. This is termed as a randomized complete block design (RCBD), and is the most commonly used manipulative design. Many other types of manipulative designs have been developed for field experimentation (Steel and Torrie 1980) and the advice of a biometrician is invaluable for the design of these types of experiments.

1.5.2 Soil Sampling for Nutrient Inventories

These are a particular type of inventory study that are undertaken to provide average values of soil nutrient properties over a field or field segment (more commonly called soil testing). This average value is then often used as the basis for fertilizer recommendations in the next growing season. The accuracy with which soil test results reflect the true condition of soils in the field is more dependent on the way in which the sample is collected and handled rather than on error associated with the laboratory analysis (Cline 1944; Franzen and Cihacek 1998). As such, the sample used for laboratory analysis must be representative of the field from which it was taken and sample collection and sample handling must not cause a change to the soil properties of interest before the laboratory analysis.

The development of a sampling procedure must address the following points.

Division of the Field into Different Sampling Units

The farm operator must decide what level of detail is relevant to his or her field operations. Are there parts of the field that have different fertility patterns? Are these areas large enough to be relevant? Does the operator want to engage in site-specific management? Has the operator has the ability to vary fertilizer application rates to accommodate the field subsections identified?

Subsections of a field would commonly be identified by differences in topography (termed *landscape-directed soil sampling*), parent material, management history, or yield history. It may be impossible to subdivide a field into smaller units if the farm operator has no prior knowledge of the field, or if there is no obvious topographic or parent material differences. Under these conditions a grid sampling design has the potential to provide the greatest amount of spatial detail. However, a grid is also the most expensive sampling method and is not typically economically feasible for routine soil testing.

Where landscape-directed soil sampling can be implemented it has been shown to provide superior information on nutrient distribution and the identification of separate management units than that obtained via grid sampling. Landscape-directed soil sampling is particularly effective at assessing patterns of mobile soil nutrients.

Selection of Sampling Design and Sample Numbers

For each field or field subsection samples can be taken using a random sampling design, a grid sampling design, or a benchmark sampling design.

In random sampling individual samples are collected from locations that are randomly distributed across the representative portion of the field. These random locations can be generated with a GPS. A zigzag sampling pattern (Figure 1.4) is often used for field sampling. The sampler should avoid sampling atypical areas such as eroded knolls, depressions, saline areas, fence lines, old roadways and yards, water channels, manure piles, and field edges. Typically, all samples are combined and a composite sample is taken and submitted for laboratory analysis. Composite sampling is comparatively inexpensive since only one sample from each field or subsection of a field is sent for laboratory analysis. However, this design provides no assessment of field variability, and relies on the ability of the farm operator to identify portions of the field that may have inherently different nutrient levels.

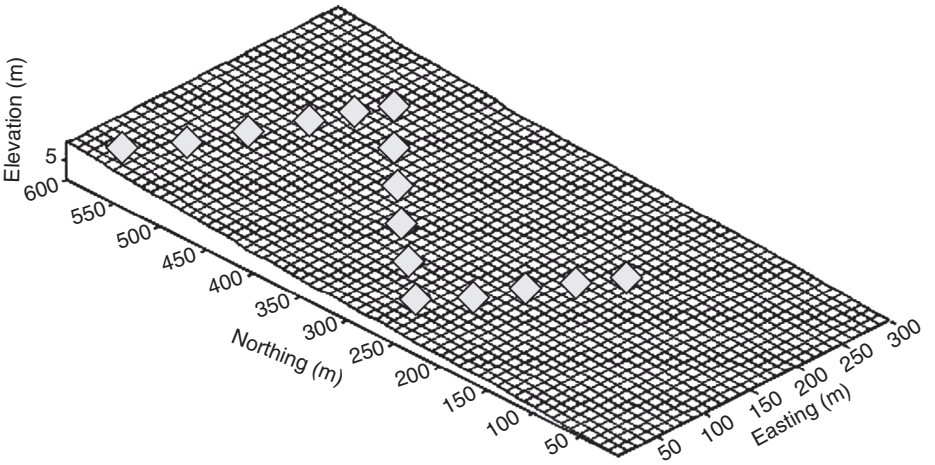

FIGURE 1.4. Example of a zigzag sampling layout on a near-level surface. Soil samples would be taken at each point labeled with a diamond shape.

Soil-testing laboratory guidelines consistently suggest that on average 20 samples be collected for each field or subsection of a field regardless of the actual area involved.

Grid Sampling

In this sampling design a grid system is imposed over each field or subsection of a field. One composite sample from each grid node is sent for laboratory analysis. The grid sampling design is the most expensive method employed in soil sampling but it can provide highly detailed information about the distribution of nutrient variability if the grid size is small enough.

Benchmark Sampling

In this design a single representative site (benchmark) is selected for each field or subsection of a field. The benchmark site should be approximately 1/4 acre or 30×30 m. Twenty or more samples should be randomly taken from within the benchmark and then composited. The farm operator can return to the same benchmark site in subsequent years for repeated testing. The advantage of this design is that year to year changes in nutrient status are more accurately reflected.

1.5.3 Sample Timing, Depth of Sampling, and Sample Handling

As a general rule, sampling for mobile nutrients should be taken as close to seeding as possible or when biological activity is low. Fall sampling should generally start after the soil temperature is less than 10°C at which time no further changes in the soil nutrient levels are expected. Spring sampling, before seeding, can be done as soon as the soil frost is gone.

Commonly used sample depth combinations are 0 to 15 cm (0″–6″) and 15 to 60 cm (6″–24″), or 0 to 30 cm (0″–12″) plus 30 to 60 cm (12″–24″). However, if the soil nutrient of interest is expected to be stratified by depth, as with water-soluble highly mobile nutrients, then additional sampling increments would help ensure accurate recommendations. If organic matter and/or pH measurements are of importance (particularly when evaluating potential herbicide residue carryover) then a 0 to 15 cm (0″–6″) sample should be taken.

To ensure that a uniform volume of soil is taken through the full depth of each sampling increment samples should be collected using soil probes and augers designed for this purpose. A wedge-shaped sample like that collected using a spade will not give consistent results. All probes should be kept clean and rust free. Avoid contamination at all stages of sample handling.

In many situations, a lubricant will need to be applied to the soil probe to prevent the soil sticking inside the probe. This lubricant will help to prevent compaction of the soil as the probe is pressed into the ground, and it will facilitate emptying the collected sample from the probe. Research by Blaylock et al. (1995) suggests that the commonly used lubricants will not affect soil test results other than the case of the micronutrients iron, zinc, manganese, and copper. The most commonly used lubricants include WD-40 lubricant, PAM cooking oil, and Dove dish-washing liquid.

1.5.4 Sampling for Geostatistical, Spectral, and Wavelet Analysis

The choice of geostatistical techniques over the approaches discussed above involves a fundamental decision about whether the sampling is design based or model based; potential users of the geostatistical approach are referred to Brus and de Gruijter (1997) (and the discussion papers following their article) and de Gruijter (2002) for a comprehensive discussion of the difference between the two approaches.

Geostatistics, spectral analysis, and wavelet analysis all address the spatial dependence in soil properties between locations. Thus the location of each sample point in space using GPS-determined spatial coordinates is critical information. Sample programs where this type of analysis is intended should include a topographic survey and generation of digital elevation model.

Sampling for Geostatistics

Spatial variability in soil properties can be separated into random and nonrandom components (Wilding and Drees 1983). The nonrandom variability is due to the gradual change of a soil property over distance. Knowledge of this nonrandom variation gained through the application of geostatistics can be useful in the design of efficient sampling programs and the estimation of the value of a soil property at unsampled locations. There are comprehensive discussions of geostatistics in Webster and Oliver (1990), Mulla and McBratney (2000), and Yates and Warrick (2002).

Geostatistics assume that the value of a soil property at any given location is a function of the value of that same property at locations nearby (spatial dependence). The distance and direction between locations determine the degree of spatial dependence between values of a soil property at those locations. The use of geostatistics thus requires that not only the value of a soil property be known, but the location as well. The primary geostatistical tools are the semivariogram and kriging. The semivariogram provides a measure of spatial dependency, the range, which can be used to determine optimum sample spacing or the extent of soil unit boundaries. Kriging is used to estimate the value of a soil property at a location where the value is unknown by using the known values at locations about the point of interest. Spatial dependence between two different soil properties can be explored using cross-semivariograms and cokriging techniques.

A common sample design to determine optimal sample spacing and soil boundary definitions is the linear transect. Calculations are simplest if equal spacing is maintained between

sample points; however, unequal spacing can be accommodated with more complicated mathematics. If the study area has recognizable topographic features then the transect should be directed perpendicular to the trend of these features.

Kriging techniques require that sample locations are taken on a grid. Sample locations are typically chosen by random selection from a set of predetermined grid intersections. In this case distances between locations are not equal. Efficient grid design and kriging may be based on a semivariogram constructed from preliminary sampling along a transect in the same area.

Geostatistics require the assumption of stationarity. Stationarity assumes that all values of a soil property within an area are drawn from the same distribution. This assumption is not always valid. As well, variation in a soil property may occur at more than one scale. For scale analysis and nonstationarity more advanced statistical techniques must be used.

Sampling for Spectral Analysis

In landscapes where landforms are repetitive such as a hummocky, rolling, or undulating terrains the continuous variation of soil properties may result in a data series with a repetitive cycle of highs and lows. The periodicity may be examined in the frequency domain using techniques referred collectively as spectral analysis (see McBratney et al. 2002 for a recent discussion of these techniques). The total variance of a data series is partitioned by frequency. The soil property is considered to cycle at a particular period if a significant portion of the variance is associated with the frequency represented by that period. Period is comparable to scale or distance much like the range from a semivariogram. Unlike a semivariogram, more than one scale can be identified. A cross spectrum can identify soil properties that cycle together and the coherency spectrum can identify scales at which two properties may be positively or negatively correlated in the same area.

The linear transect is the most common sample design used to amass a data series for spectral analysis. Sample spacing must be consistent. As for geostatistical methods the number of samples, the spacing, and the direction of the transect should be chosen to best represent the landscape features of the site.

Sampling for Wavelet Analysis

Both geostatistics and spectral analysis require the assumption of stationarity. Nonstationarity can occur, for example, due to changes in land use or geomorphology across the site, resulting in more than one population of values. A method of analysis that does not require the assumption of stationarity is wavelet analysis (see McBratney et al. 2002; Si 2003 for recent summaries of developments in this technique). A wavelet is a mathematical function that yields a local wavelet variance for each point in a data series. Like spectral analysis, wavelets portion the total variance of a data series according to frequency (scale), but unlike spectral analysis the total variance is also portioned according to space (location). A wavelet approach allows the ability to discern between multiple processes occurring in the field, the scale at which the processes are operating, and the location or distribution of these processes along the data series.

Like spectral analysis, wavelet analysis requires a data series collected from locations spaced equally along a linear transect. Wavelets are rescaled by powers of two and thus transects that contain a power of two data points (64, 128, 256, ...) are best for computational speed (Si 2003). As a result, large transects are common when using

wavelet analysis. In cases where the number of transect locations is not a power of two, the data series can be padded with zero values to the nearest power of two. Transects of 128 points are large enough for detailed scale analysis, yet may be manageable by most research programs.

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Chapter 2
Sampling Forest Soils

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2.1 INTRODUCTION

The causes for forest soil variability are many. Spatial variability is a function of bedrock type and parent material, climate, tree species composition and understory vegetation, disturbances (e.g., harvesting, fire, windthrow), and forest management activities (e.g., site preparation, thinning, pruning, fertilization, vegetation management). For example, a second generation 50-year-old Radiata pine plantation grown on plowed alluvial sands in Australia would have lower spatial variability compared to mixed hardwoods developed from a shallow rocky till of the Precambrian (Canadian) Shield after harvest. The mixed hardwoods would likely show high variability in forest floor properties such as forest floor thickness due to tree fall (Beatty and Stone 1986; Clinton and Baker 2000) and the influence of different tree species (Finzi et al. 1998; Dijkstra and Smits 2002). Moreover, the fact that the soil is plowed in the pine plantation would likely reduce some of the soil variability that could have been created by the previous plantation (e.g., changes in soil properties when sampling away from the stem). In the mineral soil, it would be more difficult to assess nutrient pools compared to the pine plantation because of the problem of measuring bulk density and percentage of coarse fragments in the rocky till (Kulmatiski et al. 2003). It would also be more problematic to develop a replicated sampling scheme by depth in the natural forest because horizon thickness across the landscape evolves as a continuum with complex spatial patterns (e.g., Ae pockets along old root channels and thick FH material in pits).

All these sources of spatial variability must be considered in efforts to systematically sample and describe forest soil properties. This is why sampling strategies and methodologies must be selected with care and this chapter is dedicated to that goal; however, information regarding field designs and plot establishment can be found in Pennock (2004) or Pennock et al. (see Chapter 1).

2.2 SAMPLE SIZE

Developing a sampling scheme that represents the inherent variability and true value of the population mean in forest floor chemistry may require many sampling points. Calculating the sample size is important because a sample size that is too large leads to a loss of time, human

resources, and money, whereas a sample size that is too small leads to erroneous statistical testing. The margin of error (d) is the maximum difference between the observed sample mean and the true population mean. It can be calculated according to the following equation (Snedecor and Cochran 1980):

$$d = t_{\alpha}^{2} \frac{s}{\sqrt{n}} \tag{2.1}$$

where t_{α} is the Student t factor for a given level of confidence (generally 95%) and s is the coefficient of variation (CV) as a percentage of the mean value. The equation can be rearranged to solve the sample size needed to produce results to a specified p and margin of error:

$$n = \left[\frac{t_{\alpha} s}{d}\right]^{2} \tag{2.2}$$

In a field study designed to test the spatial variability of nutrient concentrations and pools in the forest floor, Arp and Krause (1984) sampled the forest floor at 98 locations in a 900 m^2 plot. They showed that concentrations and pools of KCl extractable NO_3-N and NH_4-N and extractable P on field-moist soils had the highest CV values and required as many as 1371 samples (i.e., KCl extractable NO_3-N pool) to decrease the margin of error on the population mean to 10% at a confidence level of 95% and $t_{\alpha} = 1.96$ ($\alpha = 0.05$). An accurate estimate of the mean content of a nutrient required more samples than that for measuring its mean concentration. This was due mostly to the large variation in forest floor weight and thickness in the study. Figure 2.1 shows margins of error obtained using CV values in Arp and Krause (1984) with 10, 15, and 20 sampling points and confidence level set at 95%. This simple exercise demonstrates that a margin of error of 5% is generally not possible using 10 sampling points, except for total C concentration and soil pH. For nutrient concentrations (except for NO_3-N, NH_4-N, and P on field-moist soils) and physical properties (i.e., moisture, thickness, and weight), a margin of error between 31% and 9.9%, 26% and 8.0%, and 22% and 7.0% is possible with 10, 15, and 20 sampling points, respectively, with forest floor weight having the highest margin of error and total N having the lowest. However, 20 sampling points are required to obtain a margin of error between 19% and 29% when these concentrations are transformed as pools. Similarly, McFee and Stone (1965) found that it was necessary to have 50 sampling points to have a 10% margin of error (confidence level of 95%) on the calculated mean of forest floor weight and thickness for forest plots in the Adirondacks. This supports the idea that the problem of assessing forest floor nutrient pools with a high level of confidence comes in large part from the high variability in forest floor weight and thickness. Results also show that it is not financially and logistically feasible to develop replicated field design testing treatment effects on concentrations and pools of KCl extractable NO_3-N and NH_4-N as well as water-extractable P pools on field-moist samples.

The number of sampling points required for a reliable representation of a plot's mean does not appear to be related to its size. Quesnel and Lavkulich (1980) and Carter and Lowe (1986) had smaller study plots (300 and 400 m^2, respectively) than Arp and Krause (1984), but the intensities of sampling required for obtaining a reasonable estimate of the plot's mean were similar. Interestingly, Carter and Lowe (1986) conducted the study with LF and H horizons as distinct samples and found that the LF horizons required fewer samples (3 to 10) than the H horizons (3 to 38 samples) for a reliable estimate of the population mean for total C, N, P, and S concentrations and pH (margin of error of 10% at a confidence level of 95%).

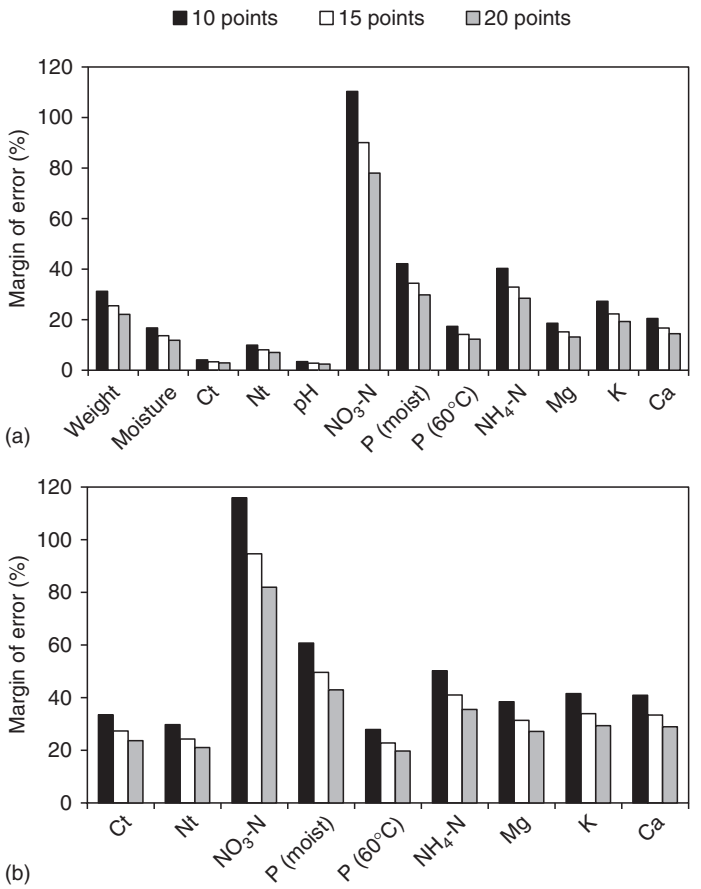

FIGURE 2.1. Margins of error of the population mean (forest floor (a) weight, moisture, pH and extractable nutrient, total C (Ct), and total N (Nt) concentrations as well as (b) extractable nutrient, Ct and Nt pools) obtained using coefficients of variation in Arp and Krause (1984) with 10, 15, and 20 sampling points with the level of confidence set at 0.95.

The results also suggested that 15 sampling points should be enough to characterize the population mean of total Mg, K, N, P, C, Cu, and Zn concentrations, lipid concentrations, pH and bulk density in LF, and H material within a margin of error of 20% at a confidence level of 95%. However, a more intensive sampling strategy was required for obtaining similar margins of error on the population mean of total Ca and Mn concentrations in the H material (81 and 47 samples, respectively) and total Al and Fe concentrations in LF material (41 and 50 samples, respectively).

In the mineral soil, the intensity of sampling required to obtain a reliable estimate of the population mean also appears to depend on the variable tested. Studying the variability of organic matter in the forest floor and mineral soil in a Tuscany forest, Van Wesemael and Veer (1992) sampled six 2500 m^2 plots and found that between 17 and 80 sampling points were required to have a 10% margin of error on the plots' population means (confidence level of 95%) of organic matter content in the first 5 cm of mineral soil compared to 33 to 235 sampling points for organic matter content in LF or FH horizons. This appears to fit with

the values of Arp and Krause (1984) who found that 114 samples were required to arrive at the same level of confidence for total C content in the forest floor. An accurate measure of the mean for soil pH, particle size, and moisture appears to be considerably easier: Ike and Clutter (1968) demonstrated that 1 to 12 sampling points in forest plots of the Georgia Blue Ridge Mountains were necessary to obtain a 10% margin of error on the population mean of pH, separate sand, silt and clay fractions, and available water and moisture. However, available P and exchangeable K concentrations required 15 to 32 samples per plot for the same margin of error, 14 to 76 samples per plot for exchangeable Mg concentration, and 153 to 507 for exchangeable Ca concentration.

2.3 SAMPLING METHODS

There are two generally accepted techniques for sampling the forest floor: soil cores or a square template. McFee and Stone (1965) used a sharp-edged steel cylinder with a diameter of 8.7 cm (59 cm^2) for coring the forest floor to quantify the distribution and variability of organic matter and nutrients in a New York podzol. Similarly, Grier and McColl (1971) used a steel cylinder with a diameter of 26.6 cm (556 cm^2). As an alternative to soil corers, Arp and Krause (1984) used a square wooden sampling template of 25 $\times$ 25 cm (625 cm^2) placed on the surface of the forest floor as a cutting guide. Others have used smaller or larger cutting templates (225 to 900 cm^2) and Klinka et al. (1981) suggested using a 10 $\times$ 10 cm template. A corrugated knife used on the inside edge of the frame will generally cut through the forest floor material with no difficulty and once the sample is cut on all sides, it is relatively simple to partition it from the mineral soil. Square sampling templates can also be constructed with heavier gauge metal and sharp edges can be added to the bottom of the frame in order to push or hammer (use hard plastic hammers or mallets) the frame into the forest floor until the mineral soil is reached. The litter can then be pulled from the frame. In some cases, a wooden cap can be built for the metal frames to assist in hammering into the forest floor. We believe this a convenient way of sampling the forest floor as it allows at the same time, after the measurement of thickness and determination of wet and dry mass, a measure of bulk density and water content.

The general rule of thumb for sampling the forest floor is that the larger the surface area being sampled, the greater chances you have of reducing microsite variability in the sample once it is air-dried, cleaned for roots and other woody material, and mixed in the laboratory. Therefore, it is recommended to use a sampling scheme that will cover, individually or bulked, at least 200 cm^2.

2.4 DIFFERENTIATING BETWEEN FOREST FLOOR AND Ah MATERIAL

Sampling of forest floor horizons varies among soil scientists and there are no accepted standards for how horizons should be sampled. Generally, LFH horizons are sampled as a whole (Bock and Van Rees 2002) or samples are taken from individual (i.e., L or F or H horizon) or combinations of horizons (i.e., FH horizon) (Olsson et al. 1996; Hamel et al. 2004), depending on the objective of the study. Normally, all layers are collected together (LFH) or the litter is collected individually (L + FH) for nutrient cycling studies or individually if one is investigating specific processes such as decomposition (e.g., Cade-Menun et al. 2000).

Sampling problems can occur when trying to distinguish between H horizons and Ah horizon sequences. In forest soils with an abrupt transition between the forest floor and the mineral

soil such as those classified as mor forest floors, it is relatively simple to distinguish the forest floor from the mineral soil. However, in forest soils with Mull and sometimes Moder forest floors (i.e., Chernozems and Melanic Brunisols), the F or H horizons are often not easily discernible from the mineral Ah horizon, thus making it more difficult to sample the forest floor layers separately. The incorporation of organic matter in the mineral soil therefore introduces a bias in forest floor sampling as some of the Ah material can be incorporated in the forest floor samples. The Expert Committee on Soil Survey (1987) defines the Ah horizon as ''A horizon enriched in organic matter, it has a color value one unit lower than the underlying horizon or 0.5% more organic C than the IC or both. It contains less than 17% organic C by weight.'' If correct sampling of the forest floor is an important issue for the study, then the most appropriate way to distinguish between the FH and Ah horizons is to carry out a presampling campaign and then conduct C analyses on the samples. Running a quick and fairly reliable loss-on-ignition (LOI) test should be very informative and allow separation between forest floor and mineral soil material: organic C constitutes 58.3% of the soil organic matter content and thus, LOI should not exceed 30% on Ah samples, whereas an LOI of 30% or more is expected from forest floor material depending on the amounts of mineral soil particles, coarse fragments, and charcoal incorporated in the material. If the cost for accessing the study site is high and there is no possibility for presampling and returning to the site after LOI testing, then a second option for separating FH horizons from Ah material is to rely on color and feel. Humus forms do vary and their taxonomy can be quite complex. In this respect, the reader is directed to Klinka et al. (1981) and/or Green et al. (1993) for an in-depth description of these horizons.

2.5 BULK DENSITY AND COARSE FRAGMENTS

Soil bulk density is a commonly measured parameter in forest soil studies to assess harvesting effects on forest soil quality such as compaction induced by logging or site preparation practices (e.g., Powers 1991; Aust et al. 1995). For forests growing on glacial till of the Precambrian Shield or other rocky soils, however, the presence of large rocks and coarse fragments makes it difficult to measure soil bulk density with standard techniques. In addition, quantifying the amount of coarse fragments is important for accurately calculating nutrient pools in soils (Palmer et al. 2002; Kulmatiski et al. 2003). There are a variety of forest soil sampling techniques to assess coarse fragments and bulk density ranging from the clod, core, pit, to the sand cone technique (i.e., Page-Dumroese et al. 1999; Kulmatiski et al. 2003). The intensive approach is to excavate a sample that is larger than the largest rock in the sample (see Chapter 66 of this book for a detailed description of the excavation and sand replacement method) while the extensive approach is to collect smaller sized samples over a large area using a corer.

Page-Dumroese et al. (1999) conducted a study where two different size cores (183 and 2356 cm^3) were compared to two pit excavation methods and one nuclear source moisture gauge for calculating bulk density. They found that bulk densities measured with the two excavation methods were 6% to 12% lower than those measured with the two core measurements and the nuclear gauge method. The nuclear gauge method gave the highest values of total and fine bulk densities and the small corer method produced the most variable results. Sampling with a corer produces higher values compared to the pit methods because compaction may occur during sampling. This was more apparent at the greater depth increments, probably because some compaction likely occurred during core insertion (Lichter and Costello 1994). To prevent this, it was suggested to remove the top mineral soil with an

auger or shovel and then hammering the corer to the desired soil depth. On the other hand, Page-Dumroese et al. (1999) also argued that the smaller corer may have provided samples too small to be representative of overall soil conditions: it is possible that the small core technique underestimates total bulk density because it does not account for large rocks with high densities. The larger size corer generally produced intermediate bulk density values, although estimates were low at the greater depths sampled because of incomplete filling or soil loss at the bottom of the core sampler. The accuracy of this method is likely increased for greater soil depths as rock fragments usually augment with depth.

Similarly, Kulmatiski et al. (2003) compared the ability of the core and excavation methods for detecting a 10% change in total C and N pools in forest soils of southern New England. They found that mean total C and N contents measured from the extensive core techniques were 7% higher than those measured from the intensive pit approach, but these differences were not statistically significant. The core techniques produced lower estimates of percentage C and N and bulk densities compared to the pit technique, but the core techniques also produced lower estimates of coarse fragments and higher soil volume values. Consequently, both techniques produced very similar estimates of total N and C soil pools. The 7% divergence between mean total C pools measured using the two techniques was reduced when coarse roots were added in the calculations, whereas coarse roots were not a significant portion of the total N pools and had no impact on estimates. The results also showed little variability of total C and N pools at a depth greater than 15 cm (assessed by the pit technique), meaning that deeper nutrient pools are insensitive to environmental factors such as tree species composition and topography. Moreover, Kulmatiski et al. (2003) suggested that the extensive core approach required less than one-half of the sampling time for determining the population mean (i.e., N and C pools) compared to the intensive pit approach and that a smaller number of samples was required for a low margin of error of the population mean. They recommended the use of the core techniques to calculate total N and C contents in the upper mineral soil horizons. However, one advantage of the pit technique is that it allows direct measurement of large rock fragments in the soil. For calculating total C and N pools in deeper soils with generally greater rock fragments, Kulmatiski et al. (2003) therefore recommended to extrapolate data from the upper mineral horizons to deeper soil by building regression models developed from a few local soil pits.

2.6 SAMPLING BY DEPTH OR DIAGNOSTIC HORIZONS?

Obtaining a reliable estimate of the population mean of a specific nutrient concentration in the mineral soil probably requires less sampling points than that in the forest floor (e.g., organic matter content in Van Wesemael and Veer (1992)). The number of sampling points is also probably less if the soil is sampled by diagnostic horizon compared to sampling by depth. More variability in soil properties is expected from sampling by depth because the sample is a mixture of soil material with different properties. For example, sampling Bhf horizons of sandy Ferro-Humic Podzols means that the soil material has at least 5% organic C and 0.4% pyrophosphate-extractable Fe and Al. However, if the mineral soil is sampled by depth, e.g., 20 cm increments, then Ae material (higher in Si and lower in Al, Fe, and C than the Bhf, see Table 2.1) is bound to be incorporated with Bhf material in the first increment and Bhf and Bf/BC material will be bulked in the second increment. In a study on jack pine growth, Hamilton and Krause (1985) showed a negative relationship between the depth of the eluvial material and tree growth. In podzols, roots develop most of their biomass in the forest floor and upper B horizons and not in the Ae material (e.g., Côté et al. 1998). Sampling by 20 cm increments in well-drained forest soils with a fully developed Ae horizon means

TABLE 2.1 Total Elemental Composition (Given as Percentage of Total Soil Matrix) of Ae and Bf Horizons of Podzols Developed under Balsam Fir in the Gaspé Peninsula of Quebec (Mean ± Standard Deviation with $n = 6$)

	Ae horizon	Podzolic B horizon
SiO_2	84.5 ± 4.18	53.3 ± 7.56
TiO_2	1.17 ± 0.16	0.68 ± 0.18
Al_2O_3	4.98 ± 1.08	11.2 ± 1.99
Fe_2O_3	0.62 ± 0.15	7.06 ± 1.79
MgO	0.24 ± 0.07	0.90 ± 0.35
CaO	0.08 ± 0.02	0.12 ± 0.05
Na_2O	0.69 ± 0.09	0.83 ± 0.18
K_2O	0.92 ± 0.24	1.34 ± 0.33
P_2O_5	0.05 ± 0.01	0.24 ± 0.08
LOI[a]	6.59 ± 3.05	24.5 ± 7.52

[a] LOI is loss-on-ignition. Total elemental composition does not sum up to 100% as trace elements are not shown here.

Note: Total iron present has been recalculated as Fe_2O_3. In cases where most of the iron was originally in the ferrous state, a higher total is the result.

that the arbitrary differences in soil morphology will govern the results of the chemical analyses. In this respect, significant correlation between tree nutrition/growth and mineral soil chemistry may be masked by the fact that the sampling scheme used is not representative of the capacity factor of the actual mineral soil to provide nutrients to the trees. Also, an admixture of soil material with different properties may camouflage the response of specific soil horizons to harvesting, acid deposition, etc., as some of the material incorporated in the sample may be in steady-state with the conditions created by the disturbance whereas the other material may not.

Note that there are also clear advantages of sampling soil by depth when conducting studies on soil changes over time. One of the best conceptual examples for demonstrating the benefits of sampling by depth is a study comparing soil C pools in a natural forest with a plantation established close by. The plantation is building a new forest floor (as it was plowed) and is likely shallower than that of the natural forest. Also, the natural sequence of horizons in the plantation is obviously different from that of the natural forest to a depth of about 5–8 cm. Therefore, as the sequencing of diagnostic horizons differs between the plantation and natural forest, sampling by depth is the best option for comparing soil C pools. Due to the horizontal variability, it is strongly recommended to sample the soil evenly across the whole sampling increment: sampling only a part of the full increment will indisputably result in artifacts. Examples of studies on long-term changes in forest soil properties that required this sampling strategy can be found in Eriksson and Rosen (1994), Parfitt et al. (1997), and Bélanger et al. (2004). Moreover, the reader will find a thorough discussion on sampling strategies to study temporal changes in soil C for agricultural soils in Ellert et al. (see Chapter 3).

2.7 COMPOSITE SAMPLING

In some forests, soil variability can be enhanced by forest processes such as tree falls to create ''pit and mound'' topography. These kinds of sites need different types of sampling strategies to account for changes in microtopography. In a study on ''pits and mounds'' in New York state hardwoods, Beatty and Stone (1986) made a composite sample from four 4.5 cm or five 2 cm diameter cores (total surface area 64 and 16 cm^2, respectively) at 0.5 or 1 m intervals across the microsites. Although these samples have a small surface area, the

sampling procedure is quite accepted considering that the study is conducted at the microsite scale and that more or larger samples were likely not needed over such a small area to calculate a valid population mean. Similarly, forest soil scientists are bulking forest floor samples for studies conducted at the plot scale, i.e., a set of samples coming from the same population (plot) are carefully mixed together so that they are equal in terms of weight or volume. Obviously, this is a tedious task to do in the field and unfortunately, it is often unclear whether proper mixing is done. Preferably, samples should be stored separately and bulking should be done in the laboratory after they have been air-dried and sieved.

A disadvantage of bulking the samples in a plot is that it does not allow for the calculation of the standard deviation or CV values. In an effort to assess the precision of the variables measured by bulking forest floor samples, Carter and Lowe (1986) compared the mean nutrient contents weighted by depth and bulk density using the 15 sampling points within a plot to the values obtained from analyzing a single sample obtained by bulking these 15 samples (as a function of depth and bulk density). Values from composite samples were all within one standard deviation of the mean, except for total P and Cu concentrations in LF material. Moreover, they investigated the relationships between the weighted means and the composite sample values across the six study plots and found that they were quite strong for most variables, suggesting that bulking samples can provide good estimates of the real population mean ($r > 0.90$, except for Ca and Al concentrations in LF, and Mn and C in LF and H horizons). Similarly, Bruckner et al. (2000) investigated the impact of bulking soil samples on microarthropod abundance on a Norway spruce plantation in Austria. It was assumed that the grinding action of soil particles during mixing would injure or kill part of the population and thus underestimate the population relative to a mean weighted from samples of the population analyzed individually. However, using a special mixing procedure of the extracts, Bruckner et al. (2000) came to the conclusion that no microarthropod was lost or damaged because a large number of samples were bulked in a systematic manner and mixed in equal amounts.

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Chapter 3
Measuring Change in Soil Organic Carbon Storage

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3.1 INTRODUCTION

Organic carbon (C) must be among the most commonly analyzed soil constituents, starting with the earliest soil investigations. Already in the nineteenth century, chemists were routinely analyzing soil C (e.g., Lawes and Gilbert 1885). Initially, these analyses were done to investigate pedogenesis and to assess soil productivity, both of which are closely linked to organic C (Gregorich et al. 1997). But more recently, scientists have been analyzing soil organic C (SOC) for another reason: to measure the net exchange of C between soil and atmosphere (Janzen 2005). Indeed, building reserves of SOC has been proposed as a way of slowing the rising atmospheric CO_2 concentrations caused by burning fossil fuel (Lal 2004a,b).

Measuring SOC to quantify soil C ''sinks'' requires more stringent sampling and analyses than measuring SOC to evaluate productivity. Where once it was sufficient to measure relative differences in concentration over time or among treatments, now we need to know the change in amount of C stored in Mg C per ha. Reviews of SOC measurement typically focus on the chemical methods of determining the SOC concentrations after samples have been brought to the laboratory. Here we emphasize soil sampling procedures and calculation approaches to estimate temporal changes in SOC stocks. Uncertainties along the entire chain of procedures, from designing the soil sampling plan, to sampling in the field, to processing and storing the samples, through to chemical analysis and calculating soil C stocks need to be considered (Theocharopoulos et al. 2004).

SOC is dynamic: newly photosynthesized C is added regularly in the form of plant litter, and existing SOC is gradually decomposed back to CO_2 by soil biota. Management or environmental conditions that change the relative rates of inputs and decomposition will effect a change in the amount of SOC stored. Rates of change in SOC (typically less than 0.5 Mg C ha^{-1} $year^{-1}$) are quite small, however, compared to the large amounts of SOC often present (as high as 100 Mg C ha^{-1}, or more, in the top 30 to 60 cm soil layer). Thus changes in SOC can only be reliably measured over a period of years or even decades (Post et al. 2001). Since the distribution of SOC in space is inherently variable, temporal changes (e.g., attributable to management practices, environmental shifts, successional change) must be distinguished from spatial ones (e.g., attributable to landform, long-term geomorphic processes, nonuniform management).

Temporal changes in SOC can be defined in two ways (Figure 3.1): as an absolute change in stored C (SOC at $t = x$ minus SOC at $t = 0$), or as a net change in storage among treatments (SOC in treatment A minus SOC in treatment B, after x years). The former provides an estimate of the actual C exchange between soil and atmosphere; the latter provides an estimate of the C exchange between soil and atmosphere, attributable to treatment A, relative to a control (treatment B). Both expressions of temporal change may be available from manipulative experiments with appropriate samples collected at establishment (assesses spatial variability) and at various intervals (say 5 to 10 years) thereafter.

This chapter provides selected methods for measuring the change in C storage, either absolute or net, typically for periods of 5 years or more. To be effective, the method needs to: measure *organic* (not total) C, provide estimates of C stock change (expressed in units of C mass per unit area of land to a specified soil depth and mass), be representative of the land area or management treatment under investigation, and provide an indication of confidence in the measurements. These methods are applicable, with minor modification, to a range of scales and settings, including benchmarks sites and replicated research experiments.

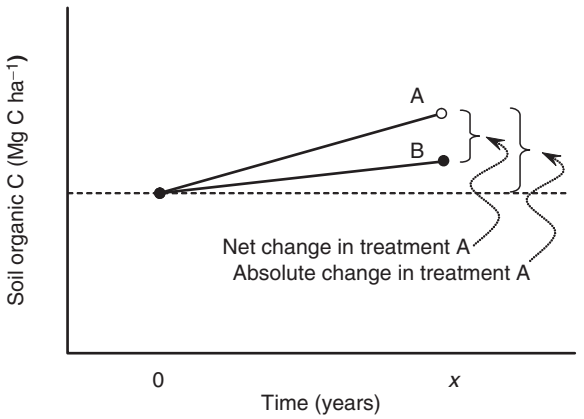

FIGURE 3.1. Illustration of hypothetical changes in soil organic C in two treatments, A and B. For treatment A, the absolute change is the difference in SOC at time = x, compared to that at time = 0. The net change is the difference between SOC in treatment A and that in treatment B, at time = x, assuming that SOC was the same in both treatments at time = 0. The latter approach is often used to measure the effect on SOC of a proposed treatment (e.g., no-till) compared to a standard ''control'' (e.g., conventional tillage).

3.2 SELECTING THE SAMPLING LOCATIONS AND PATTERN

Determining the optimum number and spatial arrangement of sampling points to estimate SOC storage remains as much an art as a science. Nevertheless, careful study of the site, along with clearly articulated objectives can improve the cost-effectiveness and precision of the estimates (VandenBygaart 2006).

3.2.1 Materials

1 Descriptions of soil properties, landscape characteristics, and agronomic history at the study site, from sources such as: soil maps and reports, aerial photos, scientific publications, cropping records, and yield maps.

3.2.2 Procedure

Two general approaches can be used in sampling a study area (e.g., a plot, field, watershed):

a Nonstratified sampling, where the entire study area is considered to be one unit, and sampled in a systematic or random manner.

b Stratified sampling, where the study area is first subdivided into relatively homogeneous units, based on factors such as topography (e.g., slope position), and each unit is sampled separately.

3.2.3 Nonstratified Sampling

1 Obtain an estimate of the likely sample variance and required accuracy for SOC at the study site, based on previously compiled information.

2 Using as much information as available, calculate the number of samples required using Equation 3.1. The required number of samples will increase as variability and the required accuracy increase (Figure 3.2) (Garten and Wullschleger 1999; Wilding et al. 2001). Required accuracy is expressed as in the same units used for the sample mean, and often is less than 10% of that value because even small changes in the mean imply appreciable pedosphere–atmosphere C exchange over large tracts of land.

3 Select an appropriate grid or linear sampling pattern, suited to the study site and sampling equipment.

3.2.4 Stratified Sampling

1 Subdivide the study site into areas likely to have similar SOC stocks, based on factors such as topography or management history.

2 Select the number of sampling sites within each subarea, using Equation 3.1, or Figure 3.2 as a guide, or by fixed allotment. In the latter case, for example, one or several sampling sites may be designated for each of three slope positions within a large research plot.

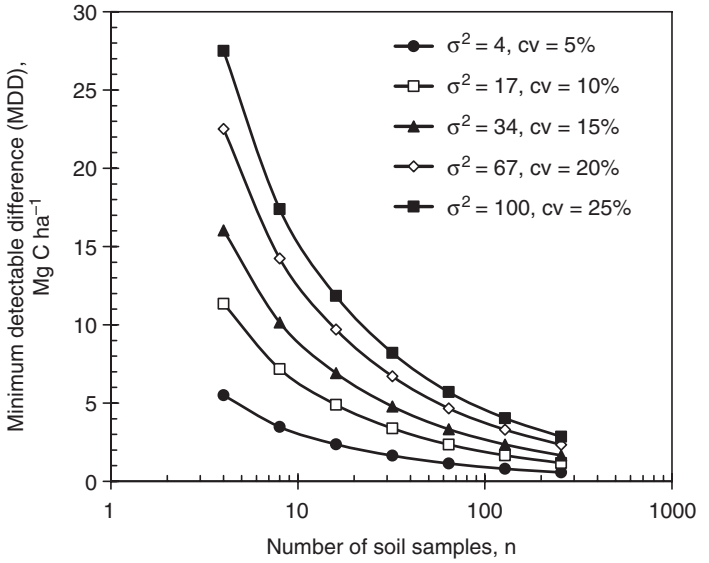

FIGURE 3.2. Decrease in the minimum detectable difference (MDD) between mean soil C at two sampling times for contrasting levels of variance as the number of samples collected at each time doubles (4, 8, 16, ...). The MDD was calculated for $\alpha = 0.05$ significance and $(1-\beta) = 0.90$ statistical power (i.e. probability of rejecting the null hypothesis when it really is false and should be rejected). The lines correspond to increasing variance (σ^2) selected for a hypothetical soil layer containing a mean of 40 Mg C ha^{-1} with the coefficient of variation (cv) increasing from 5% to 25%. (Adapted from Garten, C.T. and Wullschleger, S.D., *J. Environ. Qual.*, 28, 1359, 1999. With permission.)

3.2.5 Calculations

$$n_{\text{req}} = \frac{t^2 s^2}{(d \times \text{mean})^2} \tag{3.1}$$

where n_{req} is the required number of samples, t is the Student's t-value, at the desired confidence level (typically $1-\alpha = 0.90$ or 0.95), s^2 is the sample variance, d is the required accuracy or maximum acceptable deviation from the mean (e.g. $d = 0.10$), and mean is the arithmetic sample mean.

3.2.6 Comments

Sampling patterns and intensities will vary widely, depending on site characteristics and on other factors, notably economic considerations. Often, the number of samples required to achieve the desired sensitivity is exceedingly expensive, and the number of sampling points is somewhat arbitrarily reduced. As well, sampling intensity may have to be reduced in small plots, such as long-term experiments, where excessive soil removal may disturb the site to the extent that future research is jeopardized. But such compromises, if carried too far, may reduce the chance of measuring any differences with reasonable reliability. Studies with insufficient sampling points typically lack statistical power to assess treatment effects. Consequently, the ''cost'' of erroneous conclusions drawn from such data (when the data really are inconclusive) may greatly exceed the ''savings'' provided by reduced sample numbers.

Precisely measuring temporal changes in SOC first depends on identifying or minimizing spatial changes. Spatial changes can be minimized by pairing sampling locations in space (Ellert et al. 2001, 2002; VandenBygaart 2006). This approach allows for effective measurement of SOC changes in time at comparatively few sampling points, but measured C stock change values at these points are not necessarily representative of the entire study site. Conant and Paustian (2002) and Conant et al. (2003) have evaluated similar sampling strategies.

3.3 EXTRACTING AND PROCESSING SOIL CORES

The following procedure is intended for the extraction of soil cores, from agricultural plots or landscapes, for subsequent organic C analysis. It is provided as an illustration, recognizing that individual studies may require modification to satisfy specific objectives and local conditions.

3.3.1 MATERIALS

1 Truck-mounted hydraulic soil coring device.

2 Soil coring tube, with slots 1 cm wide by 30 cm long, and a cutting bit with inside diameter of about 7 cm. The bit usually has slightly smaller diameter (by 1 to 4 mm) than the tube; this difference should be small enough to avoid soil mixing, but large enough to prevent sticking. In dry, coarse-textured soils with weak consolidation this difference should be reduced so there is enough friction to hold the core when the tube is pulled from the soil. The diameter of the coring bit should be measured accurately and recorded for future calculations of soil core density.

3 Piston to push the soil core out of tube. A simple piston can be constructed by attaching a rubber stopper to the end of a wooden dowel.

4 Knife, steel ruler, scissors, wire brush.

5 Aluminum foil trays ($\sim 24 \times 30 \times 6$ cm, used in steam tables for serving food), coolers for transporting trays from field, and heavy polyethylene bags ($\sim 30 \times 50$ cm) to contain trays of field-moist soil.

6 Analytical balance (3000 g capacity, resolution to 0.01*g*), moisture tins (8 cm diameter $\times$ 6 cm tall), drying oven (105°C).

7 Paper ''coffee'' bags with plastic lining and attached wire ties (e.g., Zenith Specialty Bag Co., 11 $\times$ 6 cm base $\times$23 cm height).

8 ''Rukuhia'' perforated drum grinder, with 2 mm perforations (Waters and Sweetman 1955); or another coarse soil grinder and a 2 mm soil sieve.

9 Equipment to measure soil sampling locations. This may be a simple surveyor's tape to measure locations relative to permanent marker stakes in long-term field experiments, or a Global Positioning System (GPS) receiver. For precise pairing (in space) of samples collected at sequential time intervals of several years, a two-stage measuring approach may be useful: the general location is measured relative to permanent reference points or is recorded using a simple GPS receiver,

and the position of the initial cores is marked by burying an electromagnetic marker originally developed to identify underground utilities (Whitlam 1998). Alternatively, high-resolution GPS is available in many regions.

3.3.2 Procedure

1 Before sampling, label paper bags with name, sampling date, location, and soil depth. These bags, eventually to be used for storing the air-dried soils, also serve as labels throughout the sampling process. Weigh the aluminum trays, one for each sample, and record the weight on the tray.

2 In the field, for each sampling point, lightly brush away surface residue and extract a core to a depth of at least 60 cm. Move the core from the vertical to a horizontal position (e.g., in a sectioning trough made of 10 to 15 cm diameter pipe cut lengthwise), and measure the depths of any visible discontinuities (e.g., depth of A_p horizon). Be prepared to discard cores that are unrepresentative (e.g., excessively compacted during sampling, evidence of atypical rodent activity, gouged by a stone pushed along the length of the core during sampling). It may prove useful to push the core (from the deepest end) out in increments, using the top end of the tube as a guide to make perpendicular cuts. Cut the core into carefully measured segments (for example: 0 to 10, 10 to 20, 20 to 30, 30 to 45, and 45 to 60 cm), and place segments into aluminum trays, avoiding any loss of soil. Repeat the procedure for a second core, about 20 cm apart, and composite with the first core segments. Place aluminum trays inside a polyethylene bag, along with the labeled paper bag, fold over polyethylene bag, and store in cooler before subsequent processing indoors.

3 In the laboratory, remove aluminum trays from the polyethylene bags and air-dry at room temperature. Except for very sandy soils, it will be much easier to grind the soils if the field-moist soil cores are broken apart by hand before air drying and subsequent grinding. Great care is required to avoid sample losses during processing and contamination by dust, plant material, paper, or other C-rich contaminants during drying. Wear rubber gloves when handling soil to avoid contamination.

4 Once samples are air-dry, record weight of sample + aluminum tray. Remove a small, representative subsample (e.g., 50 to 80 g, excluding stones and large pieces of plant residue), and determine air-dry moisture content by oven-drying for 48 h at 105°C. Alternatively, the weights of field-moist cores plus trays may be recorded immediately after removal from the polyethylene bag and before they are broken apart and air-dried. In this case, accurate field moisture contents are crucial to estimate the densities of core segments, but spillage when cores are broken apart and mixed may be less consequential than the case when cores are dried before weighing. Thoroughly mix soils before subsampling to determine field moisture content and possibly to retain a field-moist subsample for biological analyses.

5 Crush or grind entire samples to pass a 2 mm sieve, and screen out gravel >2 mm in diameter. All organic material in the sample should be included; if necessary, separately grind roots and other large organic debris to <2 mm, and mix into the sample. A ''Rukuhia'' perforated drum grinder (Waters and Sweetman 1955)

allows efficient, effective grinding of soil samples for SOC analysis. For each sample, remove and record the air-dry weight of gravel >2 mm in diameter.

6 Place coarsely ground samples in labeled ''coffee'' bags for storage under cool, dry conditions, before analysis. For permanent storage (longer than 1 year), soil samples should be placed in sealed glass or plastic jars, and kept under cool, dry, and dark conditions. If finely ground soil is required (e.g., for elemental micro-analysis), the coarsely ground (<2 mm) soil should be thoroughly mixed and subsampled before bagging.

3.3.3 Calculations

1 Air-dry moisture content

$$W_s = (M_{AD} - M_{OD})/(M_{OD} - M_{tin}) \tag{3.2}$$

where W_s is the water content of air-dry soil, by weight (g g^{-1}), M_{AD} is the mass of air-dry soil and tin (g), M_{OD} is the mass of oven-dry soil and tin (g), and M_{tin} is the mass of tin (g).

2 Density of core segment

The following calculation provides an estimate of the density of the soil core segments. This may not be identical to more exacting estimates of soil bulk density, because compaction or loose surface layers may thwart efforts to collect samples of a uniform volume without altering the original mass *in situ*. Despite this, core segment density is preferred over a separate bulk density measurement for calculating SOC stocks.

$$D_{cs} = [(M_{cs} - M_g)/(1 + W_s)]/[L_{cs}\pi R_b^2] \tag{3.3}$$

where D_{cs} is the density of core segment (g cm^{-3}), stone-free mass averaged over the entire sample volume, M_{cs} is the total mass of air-dry soil in the core segment, M_g is the mass of gravel (g), L_{cs} is the length of core segment (cm), and R_b is the core radius (cm), i.e., inside diameter of coring bit/2. If the sample is a composite of more than 1 core segment, then L_{cs} is the cumulative length. For example, if the sample contains two segments from 10 to 20 cm depth, then $L_{cs} = 20$ cm.

3.3.4 Comments

The procedure described above may be modified to make it applicable to individual study sites and objectives. Some of the important considerations include:

a *Sampling depth*

The sampling depth should, at minimum, span the soil layers significantly affected by the management practices considered. For example, it should include the entire depth of soil affected by tillage. The preferred depth may also vary with crop type; for example, studies including perennial forages may require deeper samples than those with only shallow-rooted annual crops. As the number of sampling depths increases, so does the effort and cost of sampling, processing and analysis. Detection of a given

change in soil C (e.g., 2 Mg C ha^{-1}) becomes more difficult as the change is averaged over increasingly thick soil layers containing increasing soil C. In such instances, it may be reasonable to calculate changes for a layer thinner (to a minimum of perhaps 30 cm) than that sampled, although it might have been preferable to shift resources from sampling deeper layers to sampling at more points. The best compromise may be to sample to below the zone of short-term agricultural influence, but not much deeper. Usually, the sampling depth should be at least 30 cm for annual vegetation and 60 cm or more for perennial vegetation.

b *Division of cores into segments*

The number and length of core segments depends on the vertical heterogeneity of SOC in the profile. Generally, the greater the gradient, the shorter should be the core segments. Often, the length of segments increases with depth because the SOC is less dynamic and more uniform at depth. Where possible, core segments might be chosen to correspond roughly to clear demarcations in the profile, such as tillage depth or horizon boundary. To facilitate comparisons among a fixed soil volume it is preferable to have at least one common sampling depth, but this is not essential for comparisons among a fixed soil mass.

c *Core diameter and number per sampling point*

The preferred core diameter and number of cores per sampling point depend on the sensitivity required and the amount of soil needed for analysis. Sampling larger volumes of soil makes the sample more representative, but also increases cost and disturbance of the experimental area. Soil coring may not be feasible in stony soils that are impenetrable, but larger cores may effectively sample profiles containing some gravel.

d *Core refilling*

The soil void left after removing the sample can be filled by a soil core from an adjacent area (e.g., plot buffers), thereby preserving the physical integrity of the sampling site. This replacement, however, is labor-intensive and introduces soil from outside the treatment area which could affect subsequent samplings. Without intentional replacement, core voids become filled by adjacent topsoil, so subsequent cores should be positioned far enough away to avoid areas most affected by removal of previous cores, but close enough to exclude excessive spatial variations.

e *Core location relative to plants*

Proximity to plants may affect sample SOC contents, especially at the soil surface where plant C is concentrated at the crowns and under perennial or tap-rooted vegetation with localized plant C inputs to soil. Cores should be positioned to avoid bias, for example, when about 1/3 of the soil surface area is occupied by plants, three cores could be collected: one beneath plants, and two more between plant rows or crowns. Often basal areas occupied by the crowns of crops planted in rows are small ($\ll$30%) relative to the interrow areas, so samples are collected exclusively from the interrow. In other cases, such approximations may introduce considerable bias.

f *Measuring total soil C stocks*

In earlier studies of SOC, largely from the perspective of soil fertility, recent plant litter in the sample was often removed by sieving and discarded. In studies of C sinks, however, the total C stock should be measured. The procedure described above includes recent litter directly in the sample. An alternative approach is to analyze the plant debris separately, but include it in the calculation of C stocks. Above-ground residue, if present in significant amounts, may also need to be considered in calculating total C stocks (Peterson et al. 1998).

g *Contamination from other C sources*

Care should be taken to avoid introducing extraneous C from oil used as lubricant in soil coring tubes, wax in sample bags, and coatings on foil trays. The sample drying area should be free of dust (e.g., from plant sample processing), insects, and rodents. Cross contamination (e.g., between carbonate-rich subsoil and organic matter-rich surface soil) should be avoided during processing.

h *Repeated measurements of SOC over time*

Temporal changes in SOC can be measured with higher sensitivity if successive samples are removed from close proximity to (though not directly on) previous soil cores (Ellert et al. 2001; Conant et al. 2003; VandenBygaart 2006). To do that, the original sampling locations can be recorded using the GPS receiver, or by burying an electronic marker in one of the voids left by core removal. At subsequent sampling times, soil cores can then be taken immediately adjacent to previous cores, often in a grid pattern within ''microplots'' (Figure 3.3). The pattern may be modified to accommodate additional sampling times or other site conditions

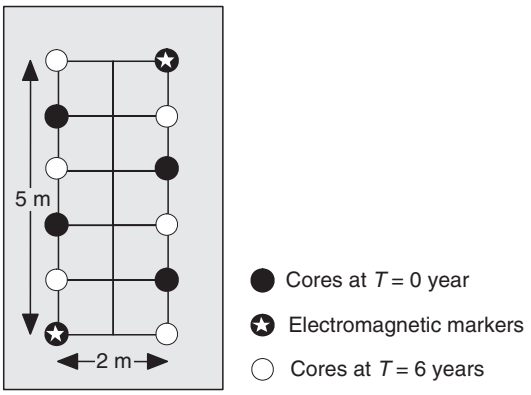

FIGURE 3.3. An example of the arrangement of soil cores within 4×7 m microplots intended for measuring temporal change in SOC stocks. (Adapted from Ellert, B.H., Janzen, H.H., and McConkey, B.G. in R. Lal, J.M. Kimble, R.F. Follett, and B.A. Stewart, (Eds.), *Assessment Methods for Soil Carbon*, Lewis Publishers, Boca Raton, Florida, 2001.)

(Conant et al. 2003; VandenBygaart 2006). To most efficiently assess temporal changes in soil C stocks, the number of cores within each microsite and of microsites within a field or plot may be adjusted for differences in variability at the microsite and field levels (Bricklemyer et al. 2005).

3.4 ESTIMATING ORGANIC C STOCKS IN SOIL

3.4.1 MATERIALS

1 Fine soil grinder and small test sieves (No. 60 with 250 μm openings and No. 100 with 150 μm openings).

2 Carbon analyzer, using dry combustion and subsequent analysis of CO_2. (For information on analysis of total and organic C see Chapter 21.)

3.4.2 PROCEDURE

1 Obtain a representative subsample of the previously stored air-dry soil samples, ideally using ''drop through'' sample riffles or centrifugal sample dividers, as needed to avoid a biased subsample. Variability introduced by simpler, more expedient approaches (e.g., small scoops from six distinct areas within a thoroughly mixed tray of air-dried, <2 mm soil) is easily quantified by collecting multiple subsamples from a few samples. Scooping from the tops of sample bags or jars is not recommended, because soil constituents tend to separate during bag or jar filling and sample handling.

2 For most microanalytical techniques the coarsely ground (<2 mm) sample will have to be finely ground using a roller or jar mill, ball-and-capsule mill, shatter-box or ring-and-puck mill, or a mortar and pestle (e.g., Kelley 1994; Rondon and Thomas 1994; McGee et al. 1999; Arnold and Schepers 2004). The preferred fineness depends on the amount of sample analyzed. If less than 0.1 g is to be combusted, the sample should be ground to pass through a 150 μm sieve. The entire subsample should be ground to pass through the designated sieve (verified by testing a representative subset of samples rather than every sample). Finely ground samples can be stored in glass vials.

3 Dry samples and standards at 60°C to 70°C for 18 h, and determine the organic C concentration (g C kg^{-1} soil) (see Chapter 21). It is critical that inorganic C be completely removed before analysis by addition of acid, or that inorganic C be analyzed separately and then subtracted from total C concentration to estimate organic C concentration (see Chapter 21). Ideally certified reference materials should be used to verify analytical accuracy, but standard soils with certified values for total and organic C remain rare (Boone et al. 1999). At minimum, standard soils prepared in-house or obtained from a commercial supplier should be used to calibrate analyses and monitor analytical precision.

4 Express the concentration in units of mg C g^{-1} dry soil (=kg C Mg^{-1} = % × 10).

3.4.3 Calculations

The SOC stock is the amount of organic C in a fixed layer of soil per unit area of land. Typically, it is expressed in units of Mg C ha^{-1} to a specified depth. Alternative units include kg C m^{-2} = Mg C $ha^{-1} \times 0.100$. The simplest way to calculate SOC stocks is to accumulate the products of concentration and core density to a fixed soil depth and volume (see calculation below). But this approach is subject to bias when comparing SOC across space or time if core density varies even slightly (Ellert and Bettany 1995). For example, when comparing SOC stocks in two treatments, if the average core density to the specified depth is 1.10 Mg m^{-3} in treatment A and 1.00 Mg m^{-3} in treatment B, then the SOC stocks in treatment A will be biased upward because it has 10% more soil in the layers compared. For that reason, SOC stocks should be calculated on an ''equivalent mass'' or ''fixed mass'' basis (see calculation below), unless core densities are very uniform.

SOC Stocks (Fixed Depth)

$$\mathrm{SOC_{FD}} = \sum_{1}^{n} D_{cs} C_{cs} L_{cs} \times 0.1 \tag{3.4}$$

where SOC_{FD} is the SOC stock to a fixed depth (Mg C ha^{-1} to the specified depth), D_{cs} is the density of core segment (g cm^{-3}), C_{cs} is the organic C concentration of core segment (mg C g^{-1} dry soil), and L_{cs} is the length of core segment (cm).

SOC Stocks (Fixed Mass)

1 For all samples, calculate the mass of soil to the designated depth:

$$M_{soil} = \sum_{1}^{n} D_{cs} L_{cs} \times 100 \tag{3.5}$$

where M_{soil} is the mass of soil to a fixed depth (Mg ha^{-1}).

2 Select, as the reference, the lowest soil mass to the prescribed depth from all sampling sites (M_{ref}).

3 Calculate the soil mass to be subtracted from the deepest core segment so that mass of soil is equivalent in all sampling sites:

$$M_{ex} = M_{soil} - M_{ref} \tag{3.6}$$

where M_{ex} is the excess mass of soil, to be subtracted from deepest core segment.

4 For each sampling site, calculate SOC stock to fixed mass:

$$\mathrm{SOC_{FM}} = \mathrm{SOC_{FD}} - M_{ex} \times C_{sn}/1000 \tag{3.7}$$

where SOC_{FM} is the SOC stock for a fixed mass of M_{ref} and C_{sn} is the SOC concentration in deepest soil core segment (mg C g^{-1} dry soil) (core segment = n).

Sample Calculations

Given the following three hypothetical soil cores:

	SOC concentration (g C kg^{-1} soil)			Density (g cm^{-3})		
Depth (cm)	Core 1	Core 2	Core 3	Core 1	Core 2	Core 3
0–10	20.0	22.0	19.0	1.04	1.10	0.99
10–20	17.4	16.3	17.1	1.17	1.27	1.20
20–40	14.3	15.2	13.9	1.30	1.35	1.25
40–60	12.2	11.9	12.1	1.40	1.45	1.42

SOC_{FD} to 40 cm is
78.3, 85.9, and 74.1 Mg C ha^{-1} for cores 1, 2, and 3, respectively.

For SOC_{FM}:
M_{soil} = 4810, 5070, and 4690 Mg ha^{-1} to 40 cm, for cores 1, 2, and 3, respectively.
Hence:
M_{ref} = 4690 Mg ha^{-1} (mass of soil core 3), and
M_{ex} = 120, 380, and 0 Mg ha^{-1}, for cores 1, 2, and 3, respectively.

Thus:
For core 1, $SOC_{FM} = 78.3 - 120 \times 14.3/1000 = 76.6$ Mg C ha^{-1}.
Similarly, SOC_{FM} = 80.1 and 74.1 Mg C ha^{-1}, for cores 2 and 3, respectively.
Thicknesses of the fixed masses $= 40 - M_{ex}/(D_{cs} \times 100)$ = 39.1, 37.2, and 40.0 cm for cores 1, 2, and 3, respectively.

Comments

The approach described to estimate SOC stocks is applicable to sites where temporal changes are attributable to biological processes (chiefly the balance between soil C inputs and outputs), rather than geomorphic processes (soil erosion and deposition). The fundamental assumption is that soil mass is largely conserved among sampling times. At sites where this does not hold, other approaches are required to estimate lateral soil redistribution or net soil imports or exports, before temporal changes in SOC may be estimated. For example at sites with considerable mass additions or removals (e.g. waste application or soil export) survey techniques that enable sampling to a fixed subsurface elevation might be appropriate (Chang et al. 2007).

Numerous variations are possible in the calculation of SOC stocks by the ''fixed mass'' approach. For example, instead of using the SOC concentration of layer n in the correction (Equation 3.7), it may be more appropriate to use the weighted mean concentration in layers n and $n+1$. Or, rather than subtracting SOC in the correction, some researchers select a reference mass and *add* SOC, based on the SOC concentration of the layer below. In all cases, the method assumes that concentration value used is representative of the layer added or subtracted. For that reason, some researchers have used core configurations with a short segment just below the depth of interest. For example, if C stocks are to be estimated for the 0 to 20 cm layer, a 20 to 25 cm segment is isolated to be used for the ''fixed depth'' calculation.

Whether comparisons are based on a fixed soil depth or mass is immaterial for situations with soil redistribution, accumulation, or export. In such situations, it is practically impossible to distinguish between the effects of geomorphological processes (soil redistribution) and biological processes (plant C inputs and SOC decay). Only in rare instances (e.g., soils with a persistent and uniform marker layer, such as a fragipan) can soil deposition or erosion be inferred from routine soil sampling.

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Chapter 4
Soil Sample Handling and Storage

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4.1 INTRODUCTION

This chapter deals with soil samples between when they are sampled and when they are analyzed. The key message is that sample handling and storage can profoundly affect analysis results, and no one way is suitable for all analytes. The issues related to soil sample handling and storage relate to the management of sample clump size, moisture content, temperature, and storage time.

With the increased availability of software to gather and interpret spatial information, there have been important advances in the past decade on methods to sample soils. Similarly, analytical capabilities have been remarkably enhanced, with greater sensitivity and more analytes. This includes notable advances in the characterization of soil organisms and biological attributes. However, there has been much less research and practical emphasis on the effects of handling and storage of soil samples. Nonetheless, there is abundant evidence that differences in handling and storage can profoundly affect the interpretation of results.

Perhaps the single most important role of analysis in soil science is to move beyond the reporting of absolutes, and toward the reporting of environmentally relevant measures. Absolute quantities, such as total elemental composition, total organic matter content, and even total porosity, are relatively simple to measure, and are relatively insensitive to effects related to sample handling and storage. However, these quantities are only partially relevant to what many researchers want to measure. Often, the more important measures are attributes such as the bioavailable or leachable elemental composition, and functional and biotic properties of the soil. For these more subtle measures, methods of sample handling and storage become critical. Examples from the literature include:

- Plant-available nitrogen (Craswell and Waring 1972; Wang et al. 1993; Verchot 1999; Fierer and Schimel 2002; Magesan et al. 2002; Riepert and Felgentreu 2002), phosphorus (Potter et al. 1991; Grierson et al. 1998; Turner and Haygarth 2003; Worsfold et al. 2005), potassium (Luo and Jackson 1985), and sulfur (Chaudhry and Cornfield 1971; David et al. 1989; Comfort et al. 1991)

- Speciation of metals and soil solution composition (Leggett and Argyle 1983; Lehmann and Harter 1983; Haynes and Swift 1985, 1991; Walworth 1992; Neary and Barnes 1993; Meyer and Arp 1994; Simonsson et al. 1999; Ross et al. 2001)

- Soil biological activity (Ross 1970; Zantua and Bremner 1975; Ross 1989; Van Gestel et al. 1993; Stenberg et al. 1998; Mondini et al. 2002; Allison and Miller 2005; Goberna et al. 2005)

- Studies of soil organic matter (Kaiser et al. 2001)

- Extraction of organic contaminants (Belkessam et al. 2005)

Without doubt, researchers must refer to the primary literature to identify the requirements and limitations for sample handling and storage specific to the analysis they undertake. It is not a default process; the researcher must be able to defend the sampling handling and storage decisions. Unfortunately, several researchers have shown that the effects of sample preparation and storage are not similar from soil to soil, so that inappropriate handling can jeopardize interpretation of results among different soils (e.g., Brohon et al. 1999; Neilsen et al. 2001).

The objective of this chapter is to provide guidance on sample handling, including compositing, reduction in clump size, and management of soil moisture. Table 4.1 gives an overview. The chapter also discusses two aspects of sample storage; storage between sampling and the primary analysis, and the long-term storage or archive of samples. Handling of samples of soil constituents separated in the field, such as soil pore water collected in lysimeters (e.g., Derome et al. 1998) is not discussed.

4.2 STEPS IN HANDLING AND STORAGE

The requirements for each sampling campaign will differ, but a typical sequence is as follows:

- Collect composite sample in the field or from the experimental system.

- If the sample is too large, reduce clump size, mix and package a portion of the composite to transport to the laboratory.

- Collect a subsample for determination of moisture content, the subsample is weighed, dried at 105°C, and reweighed.

- Dry remaining sample to a moisture content suitable for further sample handling.

- If appropriate and required, further reduce clump size, such as by grinding.

TABLE 4.1 Typical Attributes for Handling and Storage of Soil Samples

Analyte	Compositing and clump reduction	Moisture	Storage before analysis	Archival storage
Soil fauna: earthworms, nematodes, other invertebrates	Avoided, generally use minimally disturbed soil cores or clods (point samples, not composites)	Handle field-moist	Minimal time, refrigerated but not frozen	Not possible for primary analytes, suitable for some ancillary measurements
Microbial activities: respiration, functionality assays	May be minimally disturbed point samples or composites of gently ground soil	Field-moist or workable moisture content	Minimal time, refrigerated but not frozen	Not possible for primary analytes, suitable for some ancillary measurements
Microbial populations: enumeration, population types	Need for aseptic conditions often results in point samples (not composites)	Field-moist or workable moisture content	Minimal time, refrigerated but not frozen	Not possible for primary analytes, suitable for some ancillary measurements
Microbial attributes: PLFA, DNA	May be minimally disturbed point samples or composites of gently ground soil	Field-moist or workable moisture content	Varies with analysis, freezing may be appropriate	Varies with analyte, extremely low temperature freezing (−80°C) may be appropriate
Soil organic matter: structure, composition	Moderately aggressive grinding may be acceptable	Varies with analysis, may include oven drying	Varies with analysis	Varies with analysis
Bioavailability and chemical speciation	Moderately aggressive grinding may be acceptable	Workable moisture content	Minimal time, refrigerated may be ideal	Not possible for primary analytes, suitable for some ancillary measurements
Bulk physical properties: pore size distribution, bulk density	Avoided, generally use minimally disturbed soil cores or clods (point samples, not composites)	Field-moist or workable moisture content, but results reported on oven-dried basis	Indefinite if refrigerated, may change upon freezing	Indefinite if refrigerated, may change upon freezing
Mineralogical	Aggressive grinding acceptable as long as single grains are not crushed	Generally reported on an oven-dried basis	Indefinite in dried state	Indefinite in dried state
Physical: granulometry, total organic matter content	Aggressive grinding acceptable as long as single grains are not crushed	Generally reported on an oven-dried basis	Indefinite in dried state	Indefinite in dried state
Elemental analysis: total and strong-acid extractable	Aggressive grinding acceptable	Generally reported on an oven-dried basis	Indefinite as long as contamination avoided	Indefinite as long as contamination avoided

- Subsample as required for analysis.
- Prepare an archive sample.

4.3 COMPOSITING AND REDUCTION IN CLUMP SIZE

The intended outcome of compositing and reduction in clump size is to ensure the sample represents the whole. Compositing involves the gathering and mixing of a series of individual samples, typically from a series of sampling points across the landscape. Reduction in clump size is often required so that both compositing and subsampling for analysis represent a uniform material. See Schumacher et al. (1990) for detailed discussion of methods of sample splitting and subsampling.

One key issue is that the clumps be small enough that the composite sample or subsample contains a large number of them. This is a statistical issue. Allison and Miller (2005) described how variability in biological assays increased as the size of the analyzed subsamples decreases, and Liggett et al. (1984) commented that the size of subsample required to obtain consistent measurements of plutonium in soils was too large to be practical (in their case, variability among subsamples always dominated over field variation). As a general guideline, if a required composite sample is 1 kg of soil, a reasonable clump size might be $\sim$5 g (5 cm^3) or less. If a required subsample is 0.5 g, then the ''clump'' size might better be described as powder, ground as fine as practical within the limits required by the analysis. For example, Neary and Barnes (1993) and Wang et al. (1993) both recommended grinding to pass a $<$0.5 mm mesh if subsamples were to be $<$1 g.

The other key issue is that the process of breaking up the clumps does not disrupt the analytes. Some of this is self-evident; if one is sampling to measure soil macropore properties or soil fauna, then breaking up of clumps should be minimal and not aggressive. Craswell and Waring (1972) showed that grinding affected microbial mineralization rates in soil, and Neary and Barnes (1993) found that grinding, and especially mechanical grinding, affected extractable iron and aluminum concentrations. In contrast, if the analyte is total elemental concentration, quite aggressive grinding (hammer mill, mortar, and pestle) may be acceptable, as long as the grinder itself does not introduce contamination.

More controversial is the degree of grinding appropriate for measures of bioavailable element composition, or microbial attributes. As an example, tests of soil nutrient availability (soil fertility testing) were originally calibrated with soils that had very specific preparation, typically air-dried, hand-sieved to pass a 2 mm mesh, followed by volumetric (as opposed to mass-based) sampling for analysis. More aggressive drying and grinding affects the amount of nutrient removed by the selective extractants employed, increasing the extractable P by up to 165% in some soils (Turner and Haygarth 2003). Unfortunately, gentle manual preparation is expensive and, with the commercialization of soil fertility testing, more rapid and more aggressive grinding is now the norm. It is not clear if the underlying test response data have been recalibrated accordingly.

Another difficult issue in soil sample preparation is the decision of what to do with pebbles, roots, and anything else that behaves differently during sample preparation than the bulk soil matrix. Many researchers simply remove these nonconforming materials, but obviously their presence can significantly affect the interpretation of analytical results back to the field, if for no other reason than they represent a volumetric dilution of the soil matrix. As a default,

it may be an appropriate rule to remove pebbles larger than the required mesh size, but record their mass relative to the mass of the whole soil. This implies the full sample, apart from the pebbles, is ground to pass the mesh. For roots and organic debris, it may be appropriate to simply remove these as they could be considered ephemeral to the soil. For some analytes, the organic debris might be considered an important secondary subsample. This might be the case for analysis of lipophilic compounds or of fungal activities.

Subsampling organic soils and horizons can also be problematic, especially when materials such as decaying woody plants are present within the soil profile. Knife mills may be useful for grinding fibrous organic soils, if appropriate for the intended analysis.

4.4 SAMPLE MOISTURE CONTENT

The soil moisture content of stored samples is not only of importance for issues related to sample preparation (e.g., reduction of clump size) but can also profoundly affect the results of subsequent analyses. Many soils are physically impossible to handle when they are too wet, and clay soils can be very difficult to grind if they become too dry. One argument in deciding how much to dry the sample is that soils in their native setting are usually subject to wetting and drying processes, and so drying in the laboratory to moisture contents that can be found in the field seems defensible for many analytes.

The standard for measurement of soil mass is dried at 105°C for as long as required to reach a constant weight. For analyses of soil properties reported on a dry weight basis, this basis should be, and is usually assumed to be, the weight after drying at 105°C.

However, the 105°C temperature and the resultant low moisture content are very disruptive to many soil properties. It kills meso- and microbiota, denatures organic entities including soils enzymes, oxidizes some inorganic constituents, collapses clay interlayers, and can modify other soil solids. It is a suitable dryness for absolute measures such as total elemental composition and granulometric composition, and is suitable for some levels of grinding for some soils. For many other analytes, and for successful grinding of clay or organic soils, it is better to allow the soil to retain more moisture.

Nonetheless, if soil samples are not dried to 105°C and the results are to be presented per unit of soil dry weight, then the researcher should measure the soil moisture content of the soil ''as analyzed,'' and convert the results to the 105°C-dry basis. Very often, there is little difference in moisture content between air-dried and 105°C-dried, but they cannot be assumed to be equivalent.

Typical target moisture contents are:

- Field moist or ''as is'' moisture content, which can be extremely variable but necessary to avoid disruption if living organisms are to be extracted.

- Workable, a judgment by the researcher where the soil is allowed to dry to a moisture content that is typically between field capacity and air-dry, and the soil is just dry enough to allow gentle grinding, such as sieving, with no dust production. Microbial activity will be present, seeds may germinate, and refrigerated and dark storage should be considered. As the soil still contains living organisms, allowance for gas-exchange may be required, but the sample should be protected against excessive moisture loss.

Polyethylene bags may be suitable as they allow diffusion of oxygen and limit water loss. The actual moisture content should be confirmed whenever analyses are undertaken.

- Air-dried, where the soil is allowed to equilibrate with humidity in the air, resulting in soil that is nearly as dry as oven dry and can be aggressively ground (if required). Soils at this moisture content can be stored in water-permeable containers (e.g., cardboard boxes). Microbial activity is minimal and a flush of microbial activity is expected when the soil is rewetted. This is the most convenient moisture content, as long as it is consistent with the intended analyses (see examples in Table 4.1).

- Oven-dried at 105°C, where the soil is dry enough that it will accumulate moisture from the air. Soils at this moisture content must be stored in sealed containers or desiccators, and it may be necessary to redry the soils to assure they are at the required moisture content when used. The advantage of this moisture content is that it is the reference standard.

- Oven-dried to a temperature intermediate between air-dry and 105°C, which is generally a compromise between the rather slow process of air-drying and the damaging effects of 105°C. Temperatures of 30°C–40°C are arguably in the range of temperatures experienced at the soil surface in the field. Temperatures of 50°C–80°C are compromises.

Drying a soil, even at room temperature, causes a number of reactions. Living organisms either pass into a resting stage, or die. Dissolved inorganic materials will become more concentrated in the remaining pore water, and ultimately will form precipitates or perhaps gel-phase materials. Dissolved organic materials probably coagulate, both because they become concentrated and because the salt concentration of the pore water increases. Solid organic materials will deform when dry, uncover underlying mineral surfaces and may become very hydrophobic. Mineral-phase materials are generally resistant to modification until the soil becomes extremely dry or excessive heat is used.

Given these changes, it is obvious that moisture management must vary according to the required analysis (Table 4.1). Storage of air-dried or oven-dried samples is very convenient, and although dry storage will introduce gradual changes in some soil attributes, at least for the measurement of some soil chemical and physical properties these changes may be minimal. However, some types of chemical analyses are affected by drying. For example, some soil nitrogen fertility tests are influenced by drying, and as a result some commercial laboratories request soils not be dried before being sent to the laboratory. For most other large-scale operations, such as other soil fertility testing where large numbers of samples are required, air-dried or a low temperature oven-dried samples are the norm, for convenience as well as reasonable consistency.

An approach used by some to overcome the effects of drying is to rewet and incubate soil samples before analysis. The rationale is that air-drying and rewetting are natural occurrences, and so rewetting may be appropriate mitigation for the temporary effects of air-drying. Lehmann and Harter (1983) noted some recovery of copper sorption when soils were rewetted and incubated for 1 month. Haynes and Swift (1991) noted that extractability of metals could be restored with rewetting, whereas effects of drying on extractability of organic matter ‘‘was only slowly reversed following rewetting.’’

For biological, microbial, and enzyme assays, drying should generally be avoided or restricted to drying to a workable moisture content. Numerous studies have shown that drying and then rewetting the soil has a tremendous impact on biological properties, including microbially mediated soil chemical transformations (Van Gestel et al. 1993; Riepert and Felgentreu 2002). Although some studies have shown that rewetting and incubation of dried soil restores biological activity to at least some degree, it is also clear that different segments of the microbial population respond in different ways. Consequently the degree of recovery and the time taken for microbial population and functions to reestablish differs for different soils and for different microbial groups (e.g., Fierer and Schimel 2002; Pesaro et al. 2004).

4.5 EFFECTS OF TEMPERATURE AND DURATION OF STORAGE

As indicated in the introduction, there is no default storage method for all analytes and each researcher must be able to defend decisions made about sample storage. Any analysis of biological attributes or biologically mediated activities, and any analysis of volatile or labile constituents obviously require minimal storage time and specific conditions of temperature, moisture content, and container type. Analysis of nitrogen compounds and organic chemicals subject to biodegradation are notably among those where storage conditions are an issue (Stenberg et al. 1998; Rost et al. 2002).

In situations where a living soil fauna is of interest, soil samples should be stored at 5°C rather than frozen. The ability to withstand freezing temperatures in soil invertebrates is determined by a complex set of physiological and behavioral adaptations that are time dependent, so it is generally not reasonable to assume that soil samples can be safely frozen simply because the sample comes from an area subject to seasonal freezing. Edwards and Fletcher (1971) concluded that soil storage up to a week at 5°C should not cause any serious changes in the numbers of individuals or groups of soil fauna extracted from soil samples, but that after 28 day storage at 5°C, or even earlier at higher temperatures, significant changes were to be expected.

The appropriate temperature for storing soil samples required for determining microbial parameters, including the potential of the indigenous microbial flora to degrade contaminants, is controversial. Stenberg et al. (1998) concluded it was acceptable to store soils for microflora analyses at −20°C if the soils were from areas where they were normally frozen in winter. Indeed some test guidelines that measure microbial activity (e.g., OECD 2000) agree that if soils are collected from areas where they are frozen for at least 3 months of the year, then storage at −18°C for 6 months ''can be considered.'' However several other authors, including some working on soils from northern areas, stress that freezing soil samples causes significant and long-term changes in microbial abundance and activity and that certain groups are particularly sensitive to the effects of freezing (Zelles et al. 1991; Shishido and Chanway 1998; Pesaro et al. 2003). On the other hand other microbial assays (e.g., phospholipid fatty acid [PLFA]) generally require samples to be stored in a frozen state in order to minimize degradation of the fatty acids during storage.

4.6 ARCHIVAL STORAGE

Archival storage is intended to serve a number of objectives. The most immediate is to allow reanalysis of samples where the primary results are questioned. This is a form of replication of analysis. Relatedly, it is sometimes important to measure other attributes of a specific

sample in order to explain the primary results. For example, retrospective analysis of trace element content may confirm a hypothesis about differences in the initial analyses.

However, both these objectives relate to the initial reason to collect the samples. Archive samples serve other objectives as well, related to future research. An improved analytical method may become available, and reanalysis of archived samples is one way to validate the new method and relate the new and old methods. Alternatively, another research project may require a suite of soils with the specific attributes available in the archive samples.

Another key role for archived soils samples is to provide reference standards, and in the case of ecotoxicology assays to provide a diluent soil (Sheppard and Evenden 1998). Ehrlichmann et al. (1997) commented that in their reference soils, the toxicity of organic contaminants decreased with storage time, whereas the toxicity of metals increased with storage time. Riepert and Felgentreu (2002) investigated soils stored as reference soils for plant ecotoxicity bioassays, and concluded that ''soil kept as a laboratory standard under air-dried conditions over a long time period is not suitable [. . .] due to the [. . .] microbial situation,'' especially as related to nitrogen mineralization.

There is not a lot of information on how long an archive sample remains valid. Certainly samples lose biological validity fairly quickly, but will retain physical attributes such as granulometry indefinitely. In contrast, Bollen (1977) found that samples stored dry for 54 years retained their ability to respire and oxidize sulfur, some more and some less than when the samples were originally collected.

Perhaps the single most important aspect of archived soil samples, just as with any kind of archive, is the documentation. This must include provenance of the sample, collection details, preparation and storage conditions, and ideally the linkage to the researcher, and the primary analysis the researcher completed on the samples.

4.7 CONCLUSION

A review of the literature will immediately indicate that artifacts have been shown to arise from all types of soil sample handling and storage. No one protocol is suitable for all analytes. Convenient protocols such as air-drying and grinding have profound effects on physical, chemical, and biological attributes of soils. Even soil fertility testing for phosphorus and metals can be jeopardized by subtle differences in sample handling. Soil is a living material, and perhaps soil samples need the same care in handling that is afforded to tissue samples.

The most important message of this chapter is that sample handling and storage protocols are not by default. It is the responsibility of the researcher to consider and be prepared to defend the decisions taken.

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Chapter 5
Quality Control in Soil Chemical Analysis

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5.1 INTRODUCTION

In analytical work, quality can be defined as the ''delivery of reliable information within an agreed span of time, under agreed conditions, at agreed costs, and with the necessary aftercare'' (FAO 1998). The agreed conditions include specifications as to data quality objectives (DQOs), which include precision, accuracy, representativeness, completeness, and comparability. These objectives are directly related to ''fitness of use'' of the data and they determine the degree of total variability (uncertainty or error) that can be tolerated in the data. The DQOs ultimately determine the necessary quality control (QC).

Quality management systems have been developed for analytical laboratories (USEPA 2004) and there are examples of these systems in the literature (CAEAL 1999). More information can be obtained from the International Organization for Standardization (ISO 17025).

Implementation of quality management implies the next level of quality—quality assurance (QA), defined as the ''assembly of all planned and systematic actions necessary to provide adequate confidence that an analytical result will satisfy given quality objectives or requirements'' (FAO 1998). The use of QA guarantees that the delivered product is commensurate with the intended use and ensures that data have scientific credibility, and thus permits statistical interpretations as well as management decisions to be made (AENV 2004).

All sampling and laboratory activities have one target: the production of quality data that is reliable, consistent, and has a minimum of errors. Thus, to ensure the integrity of QA a system of checks are needed to establish that quality management systems are maintained within prescribed limits providing protection against ''out of control'' conditions and ensuring that the results are of acceptable quality. To achieve this, an appropriate program

of QC is needed. QC includes ''the operational techniques and activities that are used to satisfy the quality requirements or DQOs'' (FAO 1998). Producing quality data is a major enterprise requiring a continuous effort. Approximately 20% of the total costs of analysis are spent on QA and QC.

This chapter focuses on some pertinent aspects of QC in soil chemical analysis. QA topics are not discussed but QA information can be found in CCME (1993), FAO (1998), Taylor (1990), IUPAC (1997), and ISO 17025 (2005).

5.2 SOIL CHEMICAL ANALYSIS AND ITS POTENTIAL ERRORS

Determining a property or a concentration of an analyte in a soil sample follows four general steps:

1. Sample collection and handling
2. Sample shipping and transport
3. Sample preparation and analysis
4. Results data entry, handling, and reporting

Each of these steps has the potential to introduce errors into the final estimate of a property or a concentration. The careful use of tested and established protocols at each step, along with careful tracking of the samples, can help minimize, but not eliminate the errors. Table 5.1 outlines field and laboratory sources of error, while Table 5.2 indicates some corrective actions to counteract specific laboratory errors.

5.2.1 Sample Collection and Handling

Bias caused by sampling is often difficult and expensive to measure. Field spikes (samples of analyte-free media such as clean soil or sand fortified or spiked with known amounts of the target analytes) are sometimes used to assess sampling bias. Sampling errors are usually much larger than analytical errors (Jenkins et al. 1997; Ramsey 1998; IAEA 2004).

5.2.2 Contamination

Contamination is a common source of error in soil measurements (Lewis 1988; USEPA 1989). Field blanks (analyte-free media) are the most effective tools for assessing and controlling contamination. In addition, equipment rinsate blanks may also be used. Field blanks are not effective for identifying matrix interferences or for spotting noncontaminant error sources (such as analyte loss due to volatilization or decomposition). Field spikes, however, can be used for noncontaminant sources.

5.2.3 Soil Sample Storage/Preservation

Physical and chemical changes to soil samples can occur between collection and analysis. Physical changes include volatilization, adsorption, diffusion, and precipitation, while chemical changes include photochemical and microbiological degradation (Maskarinic and Moody 1988).

TABLE 5.1 Field and Laboratory Sources of Uncertainty in Chemical Analysis Data and Their Assessment

Source of error		How to assess the error
Field		
Distributional (spatial) heterogeneity	Nonrandom spatial distribution of sample components	Increase the number of individual increments required to build a representative sample. Take replicates from spatially distinct points and take a larger number of samples. Use a less expensive and less precise analytical method
Compositional heterogeneity	Arises from the complexity of the soil (clay, silt, and sand). The error inherent in using a portion to represent the whole	Increase amount of sample taken (sample mass) to represent the matrix
Sample handling	Error caused by sampling, sample handling, and preservation	Make several large composites and split them into replicates. Also, take a larger number of samples
Laboratory		
Measurement	Error from analytical measurements, including sample preparation	Split samples into replicates just before sample preparation. Splits may be sent to another laboratory for confirmatory analysis
Data handling	Faulty data handling or transcription errors	Automate data transfer, perform data verification

The following QC practices are helpful to store and preserve soil samples:

- Seal sample containers to reduce contamination and prevent water loss.
- Minimize sample container headspace to reduce loss of volatiles.
- Refrigerate or freeze samples during storage and transportation to reduce loss of volatiles and minimize biodegradation.
- Carry out extractions and digestions as soon as possible. This keeps the analyte in the resulting extraction phase (e.g., solvent or acid), thereby stabilizing the analyte. As a result, a sample extract can be held for a longer time, up to the maximum limits as specified by the method.
- Analyze samples as soon as possible.

TABLE 5.2 Corrective Action for Laboratory Sources of Error

Source of error	Corrective action
Segregation or stratification of soils on storage	Rehomogenize before subsampling for analysis
Sample or equipment contamination by the laboratory environment	Store samples, reagents, equipment separately
Sample carryover on extraction vessels or apparatus	Rinse with cleaning solution between samples
Samples weighed, processed, or analyzed out of order	Run a known reference sample at a regular interval
Inaccurate concentrations in calibration solutions	Check new standards against old before use
Sample or calibration solution mismatch	Make up standards in extracting solution used for soil samples
Drift in instrument response	Use frequent calibration/QC checks
Poor instrument sensitivity or high detection limits	Optimize all operating parameters
Faulty data handling or human transcription errors	Proofread input, automate data transfer

Source: From Hoskins, B. and Wolf, A.M., in *Recommended Chemical Soil Test Procedures for the North Central Region*, Missouri Agricultural Experiment Station, Columbia, 1998, 65–69. With permission.

5.2.4 Sample Holding Times

Holding time is the storage time between sample collection and sample analysis, in conjunction with designated preservation and storage techniques (ASTM 2004). Usually microbiological and volatiles analyses have short holding times. A holding time study involves storing replicate spiked samples for a period of time and periodically (e.g., once a day) analyzing three replicates for a specific characteristic (e.g., toxicity). The holding time is established as the time when the concentration or characteristic drops below the criterion set by the DQOs (e.g., a 10% drop). For more information, see Chapter 4 and USACE (2005).

Maximum holding times for soil samples depend on the soil type, the analyte or the characteristic being determined, storage conditions, and loss of sample integrity (Maskarinic and Moody 1988).

Results of samples not analyzed within the specific holding time are considered ''compromised'' (see Section 5.5). The actual result (e.g., concentration) is usually assumed to be equal or greater than the result determined after the holding period has expired.

5.2.5 Subsampling the Soil Sample

In most cases, the soil sample that arrives in the laboratory is not analyzed entirely. Usually only a small subsample is analyzed, and the analyte concentration of the subsample is assumed representative of the sample itself (see Figure 5.1). A subsample cannot be perfectly representative of a heterogeneous sample, and improper subsampling may introduce significant bias into the analytical process. Bias that occurs as a result of subsampling may be improved by procedures such as grinding and homogenizing the original samples (Gerlach et al. 2002). One way to detect errors due to subsampling would be to set up an experiment where one subsamples a reference material, or a material that is already well characterized.

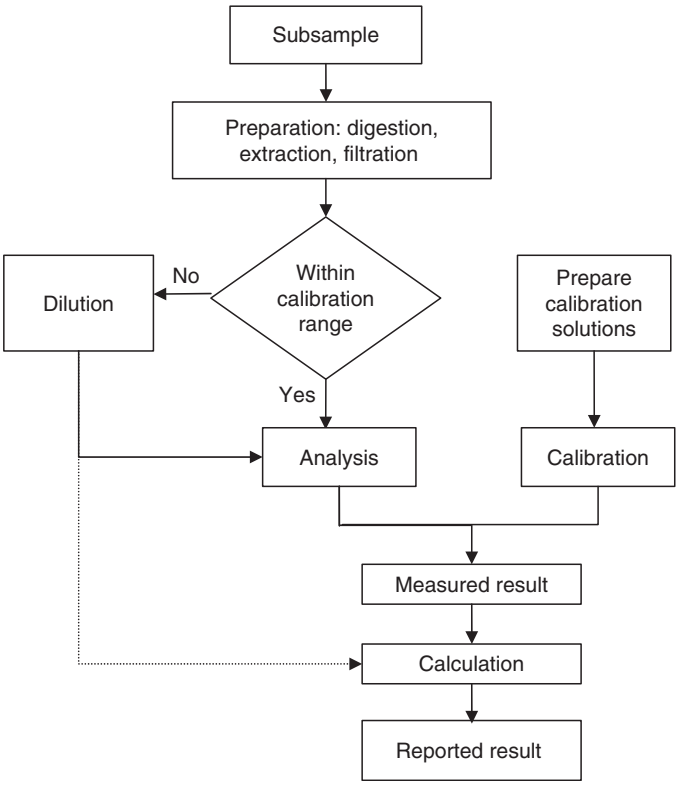

FIGURE 5.1. Laboratory sample process flow.

Once the sample enters the laboratory, it undergoes established procedures from sample preparation to final analysis. After the sample extract is introduced into the analytical instrument, the analyte is sensed by the detector and that information is converted into an electronic signal. The intensities of these electronic signals are converted into concentrations.

5.2.6 Detection Limits

Detection limits are estimates of concentrations where one can be fairly certain that the compound is present. The USEPA in 40 CFR136 (USEPA 1984) defines the method detection level (MDL) as ''the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.'' Method detection limits are statistically determined values that define how measurements of an analyte by a specific method can be distinguished from measurements of a blank (''zero'').

The MDL is a widely used precision-based benchmark of laboratory method performance determined during method validation (and periodically reevaluated). As a benchmark it compares the sensitivity and precision of various methods within and between laboratories under optimum conditions (assuming that all the laboratories determine the MDLs consistently), but it says little about the day-to-day performance of a method.

Detection limits are usually determined by analyses of replicate low-level spiked samples or blanks. A detection limit is laboratory specific as it is determined in a particular laboratory with its reagents, equipment, and analysts. Each sample will have its own detection limit,

determined by the matrix of the sample. The more the matrix interferences in the sample, the higher the sample detection limit.

One procedure to determine the MDL for an analyte is by performing seven or eight replicate analyses ($n = 7$ or 8) of the analyte at low concentration. The MDL is defined as $t \times$ sigma, where sigma is the standard deviation and t is the Student's t factor for a 99% probability level ($t = 3$ for $n = 8$). It can be reasoned that at 3 sigma concentration there is only about a 1% chance of a false positive (assuming normal distribution). Still, at the concentration of 3 sigma, there is about a 50% chance of a false negative if data are censored below that level and are treated as nondetections (see Section 5.3.2).

Interpretation of data on trace constituents (e.g., metals, organics, and pesticides) is further complicated by data censoring (not reporting concentrations below a designated limit), nondetections, and variability and bias (less than 100% recovery).

Other benchmarks besides MDL are discussed in the following sections.

Reliable Detection Limit

The reliable detection limit (RDL) is the lowest true concentration in a sample that can be reliably detected (Keith 1991). The most common definition is based on the same statistical principles as the MDL and is often defined as 6 sigma ($2 \times$ MDL), assuming sigma is constant. At this true concentration, the theoretical expected frequency of false negatives is reduced to 1% if measured values were censored at the MDL. Again, the RDL will vary from matrix to matrix and from sample to sample. For a different perspective, consult AOAC (1985), where the limit of reliable measurement is introduced.

Limit of Quantification

The concept of the limit of quantification (LOQ) is that measurements reported at or above this level meet a high standard for quantification, not just detection. Various multiples of sigma have been suggested; the higher the multiple, the greater the confidence in concentrations reported at or above this value. Commonly, the LOQ is defined at 10 sigma or $3.33 \times$ MDL. At 10 sigma, the true concentration is within $\pm 30\%$ of the reported concentration. The LOQ is equivalent to the practical quantitation limit (PQL).

Caution is advised in using method-reporting limits, because many were established using the best estimates of the analytical chemists many years ago and may have little or no statistical basis. Reporting an MDL and a limit of quantitation limit along with low-level data alerts data users of the uncertainties and limitations associated with the data. A better way would be to report $Y \pm U$ at any concentration Y found (i.e., no data censoring), where U is the calculated uncertainty at that concentration.

5.2.7 Reporting Results and Estimates of Uncertainty

A reported value from the laboratory analysis is an estimate of the true concentration in the sample at the time of collection. Thus, this measurement has variability associated with it referred to as measurement uncertainty. This uncertainty in the concentration of an analyte in a soil sample can be categorized into three general types of errors (Taylor 1988; Swyngedouw et al. 2004):

- Random errors that affect the precision of the results
- Systematic errors that affect the bias
- Blunders (mistakes that result in gross errors or lost samples—unpredictable and often yield unknown errors, i.e., the errors cannot be measured)

Although errors due to blunders are mostly controlled through proper education and training, some will always occur. Data verification and validation attempt to detect and reduce these blunders. QC samples may also detect some types of blunders.

Sampling and analytical errors do occur but are independent of each other. Therefore, sampling-related errors cannot be compensated for by the laboratory (AENV 2004). Thus, the limit of uncertainty for data on samples includes both the uncertainty of the sampling and of their measurement (Taylor 1988, 1997; Bevington and Robinson 2003) as indicated by the following equation:

$$S^2_{\text{total}} = S^2_{\text{measurement}} + S^2_{\text{sample}} \tag{5.1}$$

Estimates of uncertainty are obtained by a four-step process (Eurachem 2000):

1. Specification of the analyte
2. Identification of the uncertainty sources
3. Quantification of these uncertainty sources and
4. Calculation of the combined uncertainty

By combining uncertainty sources, only duplicate variance, long-term variance, and uncertainties in bias, calibration, and reference material need to be considered. These sources can be obtained from existing laboratory data, thus they are more easily quantified (Swyngedouw et al. 2004).

An advantage of reporting realistic estimates of uncertainty together with measurements of concentration ($Y \pm U$) is that end users of the analysis can consider the implications of the uncertainty in their use of the data. The traditional deterministic approach is to compare the measured concentration values with an appropriate regulatory threshold value. With this approach, any sampling point that has a reported concentration value below the threshold is classified as ''uncontaminated'' and those above as ''contaminated.'' This approach does not account for uncertainty in the data.

5.3 DATA QUALITY OBJECTIVES

5.3.1 Overview

DQOs specify requirements for analytical data that are clear and unambiguous concerning the intent of an investigation and the data parameters necessary to achieve that intent. These objectives are stated in both qualitative terms concerning the intended end use of the data

as well as in quantitative terms with respect to precision, accuracy, representativeness, comparability, and completeness (USEPA 2000a).

DQOs ensure that the proper methods and procedures (including method modifications) are in place with respect to MDLs, LOQs, or PQLs, applicable requirements, action limits, analyte specificity, analyte selectivity, reproducibility, false positives, and false negatives.

The following issues or stages are important for developing DQOs:

- State the precise problem to be resolved.
- Identify all the decisions needed to resolve the problem.
- Identify all the inputs needed to make the decisions.
- Narrow the boundaries of the project.
- Develop a decision rule.
- Develop uncertainty constraints.
- Optimize the design for obtaining data.

These issues are often termed the ''seven stages of DQO planning.'' Some of these stages can be further expanded as follows. Stage 1 asks ''Are the analyses primarily for characterizing the soil (e.g., pH, organic matter, texture), or for determining contaminant concentrations (e.g., metals, hydrocarbons, salts)?'' or ''Is the purpose of the soil analysis for screening or is it determinative?'' or ''Are average values of the chemicals of concern allowed?'' Chemical analyses are conducted for a purpose; hence, decisions will be made based on the analytical results. Here, one needs to consider the general kind of decisions that will be made (Stage 2). Decisions involving health and safety of the public, impacts of pollutants on the environment, regulatory compliance, and other aspects need to be considered. In Stage 3 one needs to know what analytes need to be analyzed (i.e., what are the chemicals of concern), what the associated action levels are with the decisions of Stage 2, and what detection levels need to be achieved with each analyte.

Since methods are specific for target analytes, a decision is required as to whether a particular method is appropriate or whether it will need to be modified to make it acceptable. Questions that need to be addressed involve the requirements for detection levels, method selectivity, accuracy, precision, and reproducibility (Table 5.3). These questions are addressed in the following sections.

Method Sensitivity (Detection Levels)

Estimating the lowest concentration levels needed to be achieved affects the available methods to choose from, the rates of false positive and false negative data, the ability to composite samples, and the number of samples required to meet the project DQOs.

TABLE 5.3 Recommended Method Selection and Quality Control for Different Situations

QC term	Situation	Assessment	Method selection	Alternative procedure
Sensitivity detection levels	Action level (or desired sensitivity) is close to the detection level	Need to increase the confidence levels by having precision data	Choose a bias-free method with detection levels below the action level	Increase the number of samples and field duplicates
Selectivity	Matrix effects, contamination, and interferences	Blanks for contamination, spikes, or surrogates for matrix effects and interferences	Choose a method with a specific detector that is not influenced by interferences	Run more blanks and spikes
Accuracy	Contamination, procedural losses, need bias-free data	Spikes (spiked samples analyte recovery)	Choose a bias-free method	Run more blanks, laboratory control samples, or standard reference materials
Precision	Need precision data (replicate agreement)	Need to increase confidence or decrease the standard deviations	Select a precise method	Increase replicates
Reproducibility	Multiple operators, laboratories	Interlaboratory studies	Choose an accredited and audited method	Choose another laboratory

Method Selectivity

Method selectivity directly affects the probability of detecting interferences in samples, especially in complex environmental samples. Interferences may cause an increase or decrease in signals of target analytes and thus lead to false positive or false negative conclusions. The tolerance for false positives and/or false negatives in the data is closely related to sample characteristics and method selectivity.

Accuracy

Accuracy is a measure of how close an analytical result is to its true value. It has two components, bias and precision.

Precision

To obtain overall precision (i.e., both sampling and analysis), field replicate samples need to be analyzed. Field replicate samples are two or more portions of a sample collected as close as possible at the same point in time and space to be considered identical. These samples are used to measure imprecision caused by inhomogeneity of the target analytes distributed in the soil. As imprecision increases, the relative standard deviation (RSD) will also increase. It is not unusual for the overall RSD to be larger than those of laboratory values.

Reproducibility

Reproducibility is the precision of measurements for the same sample at different laboratories, or at the same laboratory but determined by a different analyst. Reproducible results are those that can be reproduced within acceptable and known limits of deviation and therefore demonstrate correct and consistent application of standard methodologies.

5.3.2 Decision Errors

As mentioned above, two potential decision errors are identified based on interpreting sampling and analytical data.

False Positives (Decision Error B or False Acceptance)

An important criterion in chemical analytical data is ensuring that a detected parameter is present. Equally important is determining whether the mean concentration in the study area is statistically significantly higher than the action level. In either of these situations, when incorrect conclusions are made, the result is a false positive, i.e., the wrong analytes are concluded to be present. Method blanks are used to demonstrate the absence of false positives. The consequences of decision error B would result in needless expenditure of resources to pursue additional actions and assessments.

False Negatives (Decision Error A or False Rejection)

Correctly concluding from analytical data that analytes are absent from samples is also important. Failing to detect a parameter when it is present is a false negative. Similarly, concluding that a mean analyte concentration in the study area is not statistically significantly higher than the action level, when it actually is, is also a false negative. False negatives are often the result of poor recovery of analytes from soil matrices or are caused

by interferences that mask the analyte response. Method spikes (matrix spikes) are used to demonstrate the absence of false negatives. Minimization of false negatives is important with risk assessment and regulatory agencies. The consequences of decision error A would result in, for example, a health risk going undetected and unaddressed.

Both decision errors need to be examined and a decision made as to which error poses the more severe consequence. As an example, the planning team may decide that the decision error A (false negative) poses more severe consequences, because the true state of soil contamination could go undetected and may cause health risks to neighborhood residents.

Stage 6 of DQO planning sets acceptable limits for precision, accuracy, rates of false positives and/or false negative decision errors and for confidence levels in the sampling, and analytical data that relate to the DQOs. These decision error limits are set relative to the consequences of exceeding them (IAEA 2004). One could initially set the allowable decision errors to be at 1% (i.e., $P = 0.01$). This means that enough samples need to be collected and analyzed so that the chance of making either a false rejection (alpha) or a false acceptance (beta) decision error is only one out of a hundred.

5.4 QC PROCEDURES USED FOR ERROR ASSESSMENT

The type of QC samples to select depends on the DQOs of the site being investigated. Selections should be made depending on the following conditions (see Table 5.4):

- Whether bias-free and/or precision data are required.
- Whether differentiation between laboratory or sampling sources of error is needed.

TABLE 5.4 Types of Quality Control Samples Used in the Field and Laboratory

	Purpose	QC to use
Field	Check representativeness	Field duplicates (precision)
	Check for matrix effects	Surrogates, spikes, duplicates
	Check for contamination	Blanks (field blanks, rinsate blanks)
	Slowdown the chemistry	Holding times, lower temperature, appropriate containers, preservatives
Laboratory	Check representativeness	Laboratory duplicates (from subsamples)
	Check method bias	Laboratory control samples, reference materials
	Check regulations (bias)	Method detection levels (MDL), practical quantitation limits (PQL)
	Check comparability (with other laboratories)	Outside QC samples, e.g., performance test (PT) samples

Source: British Columbia Ministry of the Environment (BCME), 2003.

- Whether the degree of error to be estimated is relatively small (e.g., from typical contamination type sources) or large (e.g., from operator and/or procedural sources).

The methods selected need to be validated on soil matrices typical of those being received for analysis. Such validation does not guarantee that the methods will perform equally well for other soil types. In addition to unanticipated matrix effects, sampling artifacts, equipment malfunctions, and operator errors can also cause inaccuracies. Table 5.2 lists some sources of error that contribute to the uncertainty (variability) in analytical data.

5.4.1 Impact of Bias on Test Results

Bias is defined as the difference between the expected value of a statistic (e.g., sample average) and a population parameter (e.g., population mean). The need to take fewer replicates to reliably determine the mean value is an advantage in terms of cost and time. If no adjustment for bias is made, then for many purposes, the less biased, more variable method is preferable. However, by proper bias adjustment, the more precise method becomes the preferred method. Such adjustment can be based on QC check sample results (USEPA 2000b).

5.4.2 Field Control Samples

Field replicate, background, and rinsate (i.e., analyte-free water) blank samples are the most commonly collected field QA/QC samples for soil analysis. These are described in the following sections and are summarized in Table 5.4.

Field Replicates

Field replicates are field samples obtained from one location, homogenized and divided into separate containers and treated as separate samples throughout the remaining sample handling and analytical processes. These samples are used to assess errors associated with sample heterogeneity, sample methodology, and analytical procedures.

Equipment Rinsate Blanks

A rinsate blank is a sample of analyte-free water run over or through decontaminated field sampling equipment before collection of the next sample. It is used to assess the adequacy of cleaning or decontamination processes in the sampling procedure. The blank is placed in sample containers for handling, shipment, and analysis identical to the field samples.

Field Blanks

A field blank is a sample of analyte-free media, similar to the sample matrix, which is transferred from one vessel to another or exposed to the sampling environment at the sampling site, and shipped to the laboratory with the field samples. It is used to evaluate contamination error associated with field operations and shipping, but may also be used to evaluate contamination error associated with laboratory procedures.

Background Samples

Background samples determine the natural composition of the soil, and are considered ''clean'' samples. Although background samples are not considered QC samples per se, they are best planned for along with the QC samples. They provide a basis for comparison of, for example, contaminant concentration levels with naturally occurring levels of target analytes in the soil samples collected on site. Again, if the objective does not involve whether a site is contaminated or not, then background samples are not needed. If background samples are needed, they are collected first.

Computer expert systems are available that help researchers collect the proper type of QC samples and then calculate how many of each sample type are needed to meet the stated DQOs (Keith 2002; Pulsipher et al. 2003).

5.4.3 Laboratory QA and QC Procedures

Internal QC monitors the laboratory's current performance versus the standards and criteria that have been set, normally at the time of method development or validation.

To ensure that quality data are continuously produced during all analyses and to allow eventual review, systematic checks are performed to show that the test results remain reproducible. Such checks also show if the analytical method is measuring the quantity of target analytes in each sample within acceptable limits for bias (Environment Canada 2002a,b; USEPA 2003; IUPAC 2005). Analytical QC procedures that determine whether the sample handling procedures and laboratory methods are performing as required are presented in Table 5.5.

External laboratory QC involves reference help from other laboratories and participation in national or international interlaboratory sample and data exchange programs such as proficiency testing (PT). Such programs may involve:

- Exchange of samples with another laboratory. These samples would be prepared by a staff member other than the analyst or by the QC department. Similarly, samples prepared by the QC department can be used as internal check samples.

- Participation in interlaboratory sample exchange programs (such as round robins and/or PTs). Often in a PT study, the laboratory is not aware of samples used, in-house, for external performance evaluation.

The necessary components of a complete QA/QC program include internal QC criteria that demonstrate acceptable levels of performance, as determined by a QA review (audit). External review of data and procedures is accomplished by the monitoring activities of accreditation organizations (SCC 2005). This includes laboratory evaluation samples (PT samples, see above) and a periodic (normally every 2 years) on-site assessment of all QA/QC procedures, performed by external assessors from the accrediting organization.

5.5 DATA VERIFICATION AND REVIEW

Data verification occurs after the data analyses are completed. Data verification is a rigorous process whereby QC parameters are evaluated against a set of predetermined criteria or functional guidelines.

TABLE 5.5 Data Verification Checklist and Suggested Procedures

What to check	Why to check	How to check
Holding time (HT)	Holding times must be met for the data to be considered acceptable	Look at the chain of custody (COC) attached to the report, check the sampling date and compare this to the extraction/digestion date (or just the analysis date if no preparation step is performed) given in the report. The number of days must be less than or equal to the required HT
Blanks	Normally, only method blanks and any specific blank submitted with the samples will be reported. No blank should have a reportable concentration of any compound of interest above the reporting limit. Exceptions are the common laboratory contaminants[a]	Look at the blank reports. Any compound that has a concentration reported above the reporting limit in the blank and is present in any sample must be considered estimated or a nondetect at concentrations up to five times the level in the blank (up to 10 times for the common laboratory contaminants[a])
Surrogates	Surrogates only apply to organics at this time. Surrogates are compounds that are spiked (i.e., added at a known concentration) into every organic sample. A surrogate is a compound that is not found in nature and is not a ''normal'' pollutant	Check the report for surrogate recoveries. They should appear at the end of the analytical compounds list for a method. The recoveries should be 30%–150% to be acceptable. If the surrogate recovery is low, then flag positive values reported and reject nondetects. If it is high, then nondetect data are considered acceptable and positive data are flagged as estimated
Matrix spike (MS)/matrix spike duplicates (MSD)/ duplicates (DUP)	Spikes, spike duplicates, and duplicates are used for both organic and inorganic data. Spikes are used to check for accuracy, while duplicates are a check for precision	The MS/MSD/DUP results should appear at the end of the compound list. Verify that the recoveries are reasonable. Some values are: organic analysis (30%–150% recovery); inorganic analysis (80%–120% recovery); and duplicates (<50% relative percent difference[b])
Reporting limits	Reporting limits do not equal the method detection limits (MDLs) Reporting limits are used by laboratories as a level of confidence in reporting a concentration. Sometimes the practical quantitation limit (PQL) is used, which is 2 to 10 times the MDL	Look at the reporting limits. The limits should meet the requirements for the site. The limits for soils vary considerably depending on the method

[a] Common laboratory contaminants often include phthalates, dichloromethane, acetone, 2-butanone, hexanone, zinc, and iron.

[b] Relative percent difference (RPD) $= |X - Y| \times 200/(X + Y)$, where X and Y are the concentrations of each duplicate.

Data quality can be measured in several ways and these form the basis of deciding whether the DQOs have been met:

- Rates (%) of false positive and negatives in the analytical data
- Precision (closeness of values from repeat analyses—expressed as standard deviation)
- Bias (i.e., accuracy)
- Estimation of the uncertainty of the results

This type of information can be used to improve the quality of data interpretation. It is useful to analyze the QC data first and then review the sample data. Typical practices for analyzing QC data are presented in Table 5.5. More information on data verification is available in the literature (e.g., USEPA 1996).

5.5.1 Statistical Control

Besides documenting uncertainty, descriptive statistics from an established QA program can be used to determine if a methodology is in ''statistical control,'' i.e., whether QC criteria are being met over the long term. Check sample statistics are also used as daily decision-making tools during sample analysis to determine if expected results are being generated and if the analytical system is functioning properly (AOAC 1985). As described earlier, QC provides information to determine sample and laboratory data quality using data trend analysis (i.e., statistical process control). Statistical reports that evaluate specific anomalies or disclose trends in many areas are commonly generated (AOAC 1985; Kelly et al. 1992; FAO 1998; Garfield et al. 2000).

These trend analysis techniques are used to monitor the laboratory's performance over time, to detect departures of the laboratory's output from required or desired levels of QC, and to provide an early warning of QA or QC problems that may not be apparent from the results of an individual case.

Trend analyses also provide information needed to establish performance-based criteria for updated analytical protocols, in cases where advisory criteria were previously used (control charts).

5.5.2 Control Charts

Quality assessment statistics can be presented graphically through control charts for ease of interpretation. These charts can be used to present both bias and precision data. Repeated measurements of external or internal reference or QC samples are graphed on a time line. Superimposed on the individual results is the cumulative mean or the known value. Control levels which typically represent ± 2 sigma (upper and lower warning limits, UWL and LWL) and ± 3 sigma (upper and lower control limits, UCL and LCL) from the mean are also included (see Figure 5.2). In a normally distributed sample population, the warning levels represent a 95% confidence interval, while the control limits correspond to a 99% confidence interval. As an example, a single value outside the UCL or LCL is considered unacceptable. If statistical control is considered unacceptable, all routine sample unknowns between the unacceptable check sample(s) and the last check sample that was in control should be rerun.

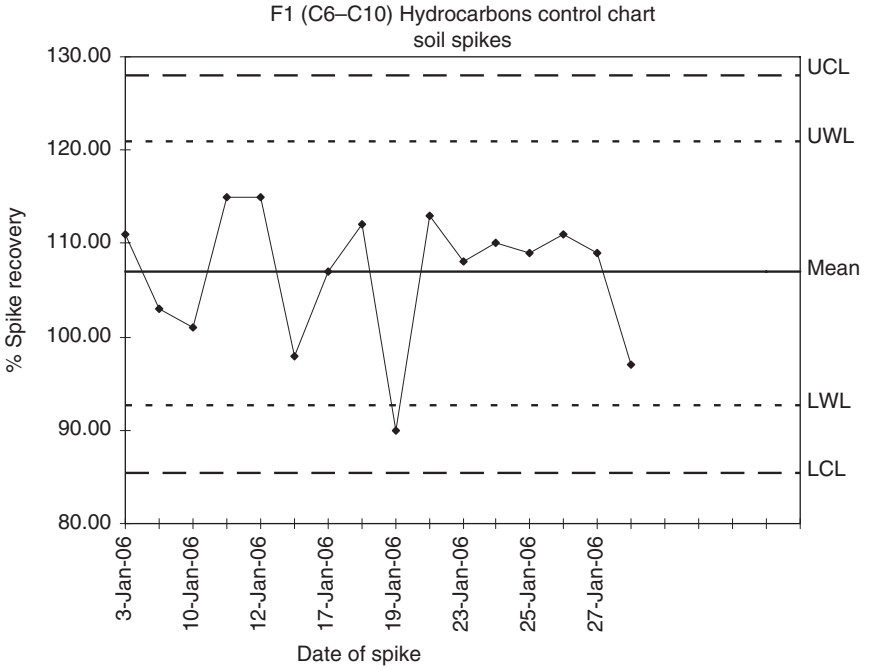

FIGURE 5.2. Example of a control chart. (UCL, upper control level, mean $+3 \times$ standard deviation of values; UWL, upper warning level, mean $+2 \times$ standard deviation of values; mean, average of values; LWL, lower warning level, mean $-2 \times$ standard deviation of values; LCL, lower control level, mean $-3 \times$ standard deviation of values.)

5.5.3 Trace of Test

When data quality is not achieved, a ''trace of test'' is a good verification tool. A systematic approach is applied in this test, starting with a check for calculation and typing errors. Items that are checked include samples, standards, reagents, equipment, glassware, and the analytical instruments and their calibrations. Then the method itself is checked, focusing on method validation factors such as sensitivity (detection limits), precision, recovery, and interferences. Batch control is also checked including laboratory control samples and reference materials used, and inspection of control charts and feedback logs (e.g., complaints).

The order of events in the investigation is the reverse of that given in Figure 5.1 and could be as follows:

1. Confirm that the results were correctly reported and correctly associated to the specific sample.
2. Recheck the results and confirm that they have been calculated correctly.
3. Verify analytical QC associated with the test to ensure the measurement process was in statistical control.
4. Investigate deviations from the routine procedure and the data record.

5 Investigate any nonconformance relating to the sample such as matrix effects and holding times.

6 Determine whether the results make sense: compare the results to other analyses, compare to historical data (if known), and/or communicate with the data user. Computer data checks can be built-in functions of laboratory databases, models, or spreadsheets. Automated QA/QC can be used to facilitate peer review or, in some cases, manual checks.

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II. DIAGNOSTIC METHODS FOR SOIL AND ENVIRONMENTAL MANAGEMENT

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Chapter 6
Nitrate and Exchangeable Ammonium Nitrogen

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6.1 INTRODUCTION

Inorganic N in soils is predominantly in the form of nitrate (NO_3) and ammonium (NH_4). Nitrite is seldom present in detectable amounts, and its determination is normally unwarranted except in neutral to alkaline soils receiving NH_4 and NH_4-producing fertilizers (Keeney and Nelson 1982). Soil testing laboratories usually determine NO_3 to estimate available N in agricultural soils, while laboratories analyzing tree nursery and forest soils often determine both NO_3 and NH_4.

There is considerable diversity among laboratories in the extraction and determination of NO_3 and NH_4. In addition, incubation methods (both aerobic and anaerobic) have been used to determine the potentially mineralizable N (see Chapter 46) and nitrogen supply rates using ion exchange resins (see Chapter 13).

Nitrate is water-soluble and a number of solutions including water have been used as extractants. Exchangeable NH_4 is defined as NH_4 that can be extracted at room temperature with a neutral K salt solution. Various molarities have been used, such as 0.05 *M* K_2SO_4, 0.1 *M* KCl, 1.0 *M* KCl, and 2.0 *M* KCl (Keeney and Nelson 1982). The most common extractant for NO_3 and NH_4, however, is 2.0 *M* KCl (e.g., Magill and Aber 2000; Shahandeh et al. 2005).

The methods of determination for NO_3 and NH_4 are even more diverse than the methods of extraction (Keeney and Nelson 1982). These range from specific ion electrode to manual colorimetric techniques, microdiffusion, steam distillation, and continuous flow analysis. Steam distillation is still sometimes employed for ^{15}N; however, for routine

analysis automated colorimetric techniques using continuous flow analyzers are preferred. Segmented flow analysis (SFA) and flow injection analysis (FIA) are continuous flow systems that are rapid, free from most soil interferences, and very sensitive.

The methods for the most commonly used extractant (2.0 *M* KCl) and SFA methods for the determination of NO_3 and NH_4 are presented here. The FIA methods often use the same chemical reactions but with different instruments (e.g., Burt 2004). The steam distillation methods for determination of NO_3 and NH_4 have not been included, since they have not changed much over the last several years. Detailed description of these methods can be found elsewhere (Bremner 1965; Keeney and Nelson 1982).

6.2 EXTRACTION OF NO_3-N AND NH_4-N WITH 2.0 *M* KCl

6.2.1 Principle

Ammonium is held in an exchangeable form in soils in the same manner as exchangeable metallic cations. Fixed or nonexchangeable NH_4 can make up a significant portion of soil N; however, fixed NH_4 is defined as the NH_4 in soil that cannot be replaced by a neutral K salt solution (Keeney and Nelson 1982). Exchangeable NH_4 is extracted by shaking with 2.0 *M* KCl. Nitrate is water-soluble and hence can also be extracted by the same 2.0 *M* KCl extract. Nitrite is seldom present in detectable amounts in soil and therefore is usually not determined.

6.2.2 Materials and Reagents

1 Reciprocating shaker.

2 Dispensing bottle.

3 Erlenmeyer flasks, 125 mL.

4 Nalgene bottles, 60 mL.

5 Filter funnels.

6 Whatman No. 42 filter papers.

7 Aluminum dishes.

8 Potassium chloride (2.0 *M* KCl): dissolve 149 g KCl in approximately 800 mL NH_3-free deionized H_2O in a 1 L volumetric flask and dilute to volume with deionized H_2O.

6.2.3 Procedure

A. Moisture determination

1 Weigh 5.00 g of moist soil in a preweighed aluminum dish.

2 Dry overnight in an oven at 105°C.

3 Cool in a desiccator and weigh.

B. Extraction procedure

1 Weigh (5.0 g) field-moist soil (or moist soil incubated for mineralization experiments) into a 125 mL Erlenmeyer flask. In some instances air-dried soil may also be used (see Comment 1 in Section 6.2.4).

2 Add 50 mL 2.0 *M* KCl solution using the dispensing bottle. (If the sample is limited, it can be reduced to a minimum of 1.0 g and 10 mL to keep 1:10 ratio.)

3 Carry a reagent blank throughout the procedure.

4 Stopper the flasks and shake for 30 min at 160 strokes per minute.

5 Filter through Whatman No. 42 filter paper into 60 mL Nalgene bottles.

6 Analyze for NO_3 and NH_4 within 24 h (see Comment 3 in Section 6.2.4).

6.2.4 Comments

1 Significant changes in the amounts of NO_3 and NH_4 can take place with prolonged storage of air-dried samples at room temperature. A study conducted by the Western Enviro-Agricultural Laboratory Association showed that the NO_3 content of soils decreased significantly after a 3-year storage of air-dried samples at room temperature (unpublished results). Increases in NH_4 content have also been reported by Bremner (1965) and Selmer-Olsen (1971).

2 Filter paper can contain significant amounts of NO_3 and NH_4 that can potentially contaminate extracts (Muneta 1980; Heffernan 1985; Sparrow and Masiak 1987).

3 Ammonium and NO_3 in KCl extracts should be determined within 24 h of extraction (Keeney and Nelson 1982). If the extracts cannot be analyzed immediately they should be frozen. Potassium chloride extracts keep indefinitely when frozen (Heffernan 1985).

4 This method yields highly reproducible results.

6.3 DETERMINATION OF NO_3-N IN 2.0 *M* KCl EXTRACTS BY SEGMENTED FLOW ANALYSIS (CADMIUM REDUCTION PROCEDURE)

6.3.1 Principle

Nitrate is determined by an automated spectrophotometric method. Nitrates are reduced to nitrite by a copper cadmium reductor coil (CRC). The nitrite ion reacts with sulfanilamide

under acidic conditions to form a diazo compound. This couples with *N*-1-naphthyl-ethylenediamine dihydrochloride to form a reddish purple azo dye (Technicon Instrument Corporation 1971).

6.3.2 Materials and Reagents

1 Technicon AutoAnalyzer consisting of sampler, manifold, proportioning pump, CRC, colorimeter, and data acquisition system.

2 CRC—activation of CRC (O.I. Analytical 2001a)—Refer to point 5 in this section for CRC reagent preparation. This procedure must be performed before connecting the CRC to the system. Do not induce air into CRC during the activation process (see Comment 6 in Section 6.3.5 regarding the efficiency of the CRC).

a. Using a 10 mL Luer-Lok syringe and a 1/4″-28 female Luer-Lok fitting, slowly flush the CRC with 10 mL of deionized H_2O. If any debris is seen exiting the CRC, continue to flush with deionized H_2O until all debris is removed.

b. Slowly flush the CRC with 10 mL of 0.5 *M* HCl solution. Quickly proceed to the next step as the HCl solution can cause damage to the cadmium surface if left in the CRC for more than a few seconds.

c. Flush the CRC with 10 mL of deionized H_2O to remove the HCl solution.

d. Slowly flush the CRC with 10 mL of 2% cupric sulfate solution. Leave this solution in the CRC for approximately 5–10 min.

e. Forcefully flush the CRC with 10 mL of NH_4Cl reagent solution to remove any loose copper that may have formed within the reactor. Continue to flush until all debris is removed.

f. The CRC should be stored and filled with deionized H_2O when not in use.

Note: Solution containing Brij-35 should not be used when flushing or storing the CRC.

Note: Do not allow any solutions other than deionized H_2O and reagents to flow through the CRC. Some solutions may cause irreversible damage to the reactor.

3 Standards

a. Stock solution (100 μg NO_3-N mL^{-1}): dissolve 0.7218 g of KNO_3 (dried overnight at 105°C) in a 1 L volumetric flask containing deionized H_2O. Add 1 mL of chloroform to preserve the solution. Dilute to 1 L and mix well.

b. Working standards: pipet 0.5, 1.0, 1.5, and 2.0 mL of stock solution into a 100 mL volumetric flask and make to volume with 2.0 *M* KCl solution to obtain 0.5, 1.0, 1.5, and 2.0 μg NO_3-N mL^{-1} standard solution, respectively.

4 Reagents

a. Dilute ammonium hydroxide (NH_4OH) solution: add four or five drops of concentrated NH_4OH to approximately 30 mL of deionized H_2O.

b. Ammonium chloride reagent: dissolve 10 g NH_4Cl in a 1 L volumetric flask containing about 750 mL of deionized H_2O. Add dilute NH_4OH to attain a pH of 8.5, add 0.5 mL of Brij-35, dilute to 1 L, and mix well. (*Note*: it takes only two drops of dilute NH_4OH to achieve the desired pH.)

c. Color reagent: to a 1 L volumetric flask containing about 750 mL of deionized H_2O, carefully add 100 mL of concentrated H_3PO_4 (see Comment 2 in Section 6.3.5) and 10 g of sulfanilamide. Dissolve completely. Add 0.5 g of *N*-1-naphthyl-ethylenediamine dihydrochloride (Marshall's reagent), and dissolve. Dilute to 1 L volume with deionized H_2O and mix well. Add 0.5 mL of Brij-35. Store in an amber glass bottle. This reagent is stable for 1 month.

5 Reagents for CRC

a. Cupric sulfate solution (2% w/v): dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in approximately 900 mL of deionized H_2O in a 1 L volumetric flask. Dilute the solution to 1 L with deionized H_2O and mix well.

b. Hydrochloric acid solution (0.5 *M*): carefully add 4.15 mL of concentrated HCl to approximately 70 mL of deionized H_2O in a 100 mL volumetric flask (see Comment 2 in Section 6.3.5). Dilute to 100 mL with deionized H_2O and mix well.

6.3.3 Procedure

1 If refrigerated, bring the soil extracts to room temperature.

2 Shake extracts well.

3 Set up AutoAnalyzer (see Maynard and Kalra 1993; Kalra and Maynard 1991). Allow the colorimeter to warm up for at least 30 min.

4 Place all reagent tubing in deionized H_2O and run for 10 min.

5 Insert tubing in correct reagents and run for 20 min to ensure thorough flushing of the system (feed 2.0 *M* KCl through the wash line).

6 Establish a stable baseline.

7 Place the sample tubing in the high standard for 5 min.

8 Reset the baseline, if necessary.

9 Transfer standard solutions to sample cups and arrange on the tray in descending order.

10 Transfer sample extracts to sample cups and place in the sample tray following the standards.

11 Begin run.

12 After run is complete, rerun the standards to ensure that there has been no drifting. Reestablish baseline.

13 Place tubing in deionized H_2O, rinse and run for 20 min before turning the proportioning pump off.

6.3.4 Calculation

Prepare a standard curve from recorded readings (absorption vs. concentration) of standards and read as μg NO_3-N mL^{-1} in KCl extract. Results are calculated as follows:

$$\text{NO}_3\text{-N in moist soil } (\mu\text{g g}^{-1}) = \frac{\text{NO}_3\text{-N in extract } (\mu\text{g mL}^{-1}) \times \text{volume of extractant (mL)}}{\text{Weight of moist soil (g)}} \tag{6.1}$$

$$\text{Moisture factor} = \frac{\text{Moist soil (g)}}{\text{Oven-dried soil (g)}} \tag{6.2}$$

$$\text{NO}_3\text{-N in oven-dried soil } (\mu\text{g g}^{-1}) = \text{NO}_3\text{-N in moist soil } (\mu\text{g g}^{-1}) \times \text{moisture factor} \tag{6.3}$$

There are data collection software packages associated with the data acquisition systems and these will automatically generate calculated concentration values based on intensities received from the colorimeter and inputs of the appropriate information (e.g., sample weight, extract volumes, and moisture factor).

6.3.5 Comments

1 Use deionized H_2O throughout the procedure.

2 *Warning*: Mixing concentrated acids and water produces a great amount of heat. Take appropriate precautions.

3 All reagent bottles, sample cups, and new pump tubing should be rinsed with approximately 1 *M* HCl.

4 Range: 0.01–2 μg NO_3-N mL^{-1} extract. Extracts with NO_3 concentrations greater than the high standard (2.0 μg NO_3-N mL^{-1}) should be diluted with 2.0 *M* KCl solution and reanalyzed.

5 Prepared CRCs can be purchased from various instrument/parts supplies for SFA systems. Previously, the method called for preparation of a cadmium reductor

column. However, preparation was tedious and time consuming and cadmium granules are no longer readily available.

6 Reduction efficiency of the CRC (O.I. Analytical 2001a).

a. In the CRC, nitrate is reduced to nitrite. However, under some conditions, reduction may proceed further with nitrite being reduced to hydroxylamine and ammonium ion. These reactions are pH-dependent:

$$NO_3 + 2H^+ + 2e \rightarrow NO_2 + H_2O \tag{6.4}$$

$$NO_2 + 6H^+ + 6e \rightarrow H_3NOH + H_2O \tag{6.5}$$

$$NO_2 + 8H^+ + 6e \rightarrow NH_4^+ + 2H_2O \tag{6.6}$$

At the buffered pH of this method, reaction 6.4 predominates. However, if the cadmium surface is overly active, reaction 6.5 and reaction 6.6 will proceed sufficiently to give low results of nitrite.

b. If the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite. This condition is defined as poor reduction efficiency.

c. To determine the reduction efficiency, run a high-level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. The reduction efficiency is calculated as given below.

$$PR = (N_3/N_2) \times 100 \tag{6.7}$$

where PR is the percent reduction efficiency, N_3 is the nitrate peak height, and N_2 is the nitrite peak height.

d. If the response of the nitrite is as expected but the reduction efficiency is less than 90%, then the CRC may need to be reactivated.

7 The method includes NO_3-N plus NO_2-N; therefore, samples containing significant amounts of NO_2-N will result in the overestimation of NO_3-N.

8 The method given in this section outlines the configuration of the Technicon AutoAnalyzer. However, the cadmium reduction method can be applied to other SFA and FIA systems.

6.3.6 Precision and Accuracy

There are no standard reference samples for accuracy determination. Precision measurements for NO_3-N carried out for soil test quality assurance program of the Alberta Institute of Pedology (Heaney et al. 1988) indicated that NO_3-N was one of the most variable parameters measured. Coefficient of variation ranged from 4.8% to 30.4% for samples with 67.3 $\pm$ 3.2 (SD) and 3.3 $\pm$ 1.0 (SD) μg NO_3-N g^{-1}, respectively.

6.4 DETERMINATION OF NH_4-N IN 2.0 *M* KCl EXTRACTS BY SEGMENTED FLOW AUTOANALYZER INDOPHENOL BLUE PROCEDURE (PHENATE METHOD)

6.4.1 Principle

Ammonium is determined by an automated spectrophotometric method utilizing the Berthelot reaction (Searle 1984). Phenol and NH_4 react to form an intense blue color. The intensity of color is proportional to the NH_4 present. Sodium hypochlorite and sodium nitroprusside solutions are used as oxidant and catalyst, respectively (O.I. Analytical 2001b).

6.4.2 Materials and Reagents

1 Technicon AutoAnalyzer consisting of sampler, manifold, proportioning pump, heating bath, colorimeter, and data acquisition system.

2 Standard solutions:

a. Stock solution #1 (1000 μg NH_4-N mL^{-1}): in a 1 L volumetric flask containing about 800 mL of deionized H_2O dissolve 4.7170 g $(NH_4)_2SO_4$ (dried at 105°C). Dilute to 1 L with deionized H_2O, mix well, and store the solution in a refrigerator.

b. Stock solution #2 (100 μg NH_4-N mL^{-1}): dilute 10 mL of stock solution #1 to 100 mL with 2.0 *M* KCl solution. Store the solution in a refrigerator.

c. Working standards: transfer 0, 1, 2, 5, 7, and 10 mL of stock solution #2 to 100 mL volumetric flasks. Make to volume with 2.0 *M* KCl. This will provide 0, 1, 2, 5, 7, and 10 μg NH_4-N mL^{-1} standard solutions, respectively. Prepare daily.

3 Complexing reagent: in a 1 L flask containing about 950 mL of deionized H_2O, dissolve 33 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot H_2O$) and 24 g of sodium citrate ($HOC(COONa)(CH_2COONa)_2 \cdot H_2O$). Adjust to pH 5.0 with concentrated H_2SO_4, add 0.5 mL of Brij-35, dilute to volume with deionized H_2O, and mix well.

4 Alkaline phenol: using a 1 L Erlenmeyer flask, dissolve 83 g of phenol in 50 mL of deionized H_2O. Cautiously add, in small increments with agitation, 180 mL of 20% (5 *M*) NaOH. Dilute to 1 L with deionized H_2O. Store alkaline phenol reagent in an amber bottle. (To make 20% NaOH, dissolve 200 g of NaOH and dilute to 1 L with deionized H_2O.)

5 Sodium hypochlorite (NaOCl): dilute 200 mL of household bleach (5.25% NaOCl) to 1 L using deionized H_2O. This reagent must be prepared daily, immediately before use to obtain optimum results. The NaOCl concentration in this reagent decreases on standing.

6 Sodium nitroprusside: dissolve 0.5 g of sodium nitroprusside ($Na_2Fe(CN)_5 NO \cdot 2H_2O$) in 900 mL of deionized H_2O and dilute to 1 L. Store in dark-colored bottle in a refrigerator.

6.4.3 Procedure

Follow the procedure (6.3.3) outlined for NO_3-N (see Kalra and Maynard 1991; Maynard and Kalra 1993).

6.4.4 Calculation

The calculations are the same as given in 6.3.4.

6.4.5 Comments

1 Use NH_4-free deionized H_2O throughout the procedure.

2 All reagent bottles, sample cups, and new pump tubing should be rinsed with approximately 1 *M* HCl.

3 Range: 0.01–10.0 μg NH_4-N mL^{-1} extract. Extracts with NH_4 concentrations greater than the high standard (10.0 μg NH_4-N mL^{-1}) should be diluted with 2.0 *M* KCl solution and reanalyzed.

4 It is critical that the operating temperature is 50°C ± 1°C.

5 The method given in this section outlines the configuration of the Technicon AutoAnalyzer (Technicon Instrument Corporation 1973). However, the phenate method can be applied to other SFA and FIA systems.

6.4.6 Precision and Accuracy

There are no standard reference samples for accuracy determination. Long-term analyses of laboratory samples gave coefficient of variations of 21%–24% for several samples over a wide range of concentrations.

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Chapter 7
Mehlich 3-Extractable Elements

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7.1 INTRODUCTION

During the past few years, numerous techniques and methods have been developed to estimate soil nutrient availability. Among these methods, the Mehlich 3 (M3) is considered an appropriate and economic chemical method since it is suitable for a wide range of soils and can serve as a ''universal'' soil test extractant (Sims 1989; Zbiral 2000a; Bolland et al. 2003). M3 was developed by Mehlich (1984) as multielement soil extraction and is widely used, especially in agronomic studies, to evaluate soil nutrient status and establish fertilizer recommendations mainly for P and K in humid regions. The following elements can be successfully analyzed using M3 extracting solution: P, K, Ca, Mg, Na, Cu, Zn, Mn, B, Al, and Fe. The extracting solution is composed of 0.2 *M* CH_3COOH, 0.25 *M* NH_4NO_3, 0.015 *M* NH_4F, 0.013 *M* HNO_3, and 0.001 *M* ethylene diamine tetraacetic acid (EDTA). M3-extractable phosphorus (M3-P) is obtained by the action of acetic acid and fluoride compounds, while K, Ca, Mg, and Na (M3-K, M3-Ca, M3-Mg, and M3-Na, respectively) are removed by the action of ammonium nitrate and nitric acid. The Cu, Zn, Mn, and Fe (M3-Cu, M-Zn, M3-Mn, and M3-Fe) are extracted by NH_4 and the chelating agent EDTA.

Many studies have compared the M3 method to other chemical and nonchemical methods and reported significant correlations between tested methods (Zbiral and Nemec 2000; Cox 2001; Bolland et al. 2003). Indeed, M3-P is closely related to P extracted by M2, Bray 1, Bray 2, Olsen, strontium chloride–citric acid, and water (Mehlich 1984; Simard et al. 1991; Zbiral and Nemec 2002). In a study conducted in Quebec, Tran et al. (1990) reported that the amount of M3-P is approximately the same as that determined by the Bray 1 method on most noncalcareous soils. Recently, Mallarino (2003) concluded that M3 test is more effective than the Bray test for predicting corn (*Zea mays* L.) response to P across many Iowa soils with pH values ranging from 5.2 to 8.2. A good correlation was also obtained between M3-P and P desorbed by anionic exchange membranes and electroultrafiltration (EUF) techniques

(Tran et al. 1992a,b; Ziadi et al. 2001). Many studies reported a strong correlation between M3-P and plant P uptake or between M3-P and relative plant yield in a wide range of soils (Tran and Giroux 1987; Ziadi et al. 2001; Mallarino 2003). Others, however, have indicated that some alkaline extractants (i.e., $NaHCO_3$) are superior to acidic extractants (M3) when used to evaluate plant P availability (Bates 1990). Depending on the determination method used, the critical level of M3-P for most common crops is about 30 to 60 $\mu g\ g^{-1}$ (Sims 1989; Tran and Giroux 1989; Bolland et al. 2003).

In addition to its value in agronomic studies, M3-P has also been used in environmental studies as an agrienvironmental soil test for P (Sims 1993; Sharpley et al. 1996; Beauchemin et al. 2003). The concept of P saturation degree was developed and successfully used in Europe and North America to indicate the potential desorbability of soil P (Breeuwsma and Reijerink 1992; Beauchemin and Simard 2000). In the mid-Atlantic USA region, Sims et al. (2002) reported that the M3-P/(M3-Al + M3-Fe) can be used to predict runoff and leachate P concentration. In a study conducted in Quebec, Khiari et al. (2000) reported that the environmentally critical (M3-P/M3-Al) percentage was 15%, corresponding to the critical degree of phosphate saturation of 25% proposed in Netherlands using oxalate extraction method (Van der Zee et al. 1987). In Quebec, the ratio of M3-extractable P to Al (M3-P/M3-Al) has been recently introduced in the local recommendation in corn production (CRAAQ 2003). The reader is referred to Chapter 14 for a more complete description of environmental soil P indices.

In addition to P, significant correlations have been obtained between the other nutrients (K, Ca, Mg, Na, Cu, Zn, Mn, Fe, and B) extracted by the M3 solution and other methods currently used in different laboratories (Tran 1989; Cancela et al. 2002; Mylavarapu et al. 2002). Furthermore, Michaelson et al. (1987) reported significant correlation between the amounts of K, Ca, and Mg extracted by M3 and by ammonium acetate. Highly significant correlations have also been reported between M3-extractable amounts of Cu, Zn, Mn, Fe, and B and those obtained by the double acid, diethylene triamine pentaacetic acid-triethanolamine (DTPA-TEA), or 0.1 *M* HCl, Mehlich 1 (Sims 1989; Sims et al. 1991; Zbiral and Nemec 2000).

The use of automated methods to quantify soil nutrients has expanded rapidly since the early 1990s (Munter 1990; Jones 1998). The inductively coupled plasma (ICP) emission spectroscopy is becoming one of the most popular instruments used in routine soil testing laboratories. The ICP instruments (optical emission spectroscopy [OES] or mass spectroscopy [MS]) are advantageous because they are able to quantify many nutrients (P, K, Ca, Mg, and micronutrients) in one analytical process. However, there has been criticism on the adoption of ICP, especially for P, instead of colorimetric methods which have been historically used in soil test calibrations for fertilizer recommendations (Mallarino and Sawyer 2000; Zbiral 2000b; Sikora et al. 2005). Because of observed differences between P values obtained by ICP and by colorimetric methods, some regions in the United States do not recommend the use of ICP to determine P in any soil test extracts (Mallarino and Sawyer 2000). Zbiral (2000b) reported a small, but significant difference (2% to 8%) for K and Mg determined by ICP-OES and flame atomic absorption. In the same experiment, the amount of P determined by ICP-OES was higher by 8% to 14% than that obtained by the spectrophotometric method. Recently, Sikora et al. (2005) confirmed these results when they compared M3-P measured by ICP with that by colorimetric method, and concluded that further research is needed to determine if the higher ICP results are due to higher P bioavailability or analytical interferences. Eckert and Watson (1996) reported that P measured with ICP is sometimes up to 50% higher than P measured with the colorimetric methods. The reason for such differences is

explained by the fact that the spectrophotometry method determines only the orthophosphate forms of P, whereas the ICP determines the total P content (i.e., organic P as well as total inorganic P forms not just orthophosphate) present in the soil extract (Zbiral 2000a; Mallarino 2003). Mallarino (2003) reported a strong relationship between P determined by ICP method and the original colorimetric method ($R^2 = 0.84$) and concluded that M3-P as determined by ICP should be considered as a different test and its interpretation should be based on field calibration rather than conversion of M3-P measured by colorimetric method. Since automated systems are frequently employed to measure the concentration of nutrient ions in the extract and specific operating conditions and procedure for the instrument are outlined in the manufacturer's operating manual, only a manual method is described in this chapter.

7.2 MATERIALS AND REAGENTS

1 Reciprocating shaker

2 Erlenmeyer flasks 125 mL

3 Filter funnels

4 Filter paper (Whatman #42)

5 Disposable plastic vials

6 Instrumentation common in soil chemistry laboratories such as: spectrophotometer for conventional colorimetry or automated colorimetry (e.g., Technicon AutoAnalyzer; Lachat Flow Injection System); flame photometer; or ICP-OES or ICP-MS

7 M3 extracting solution:

   a. Stock solution M3: (1.5 *M* NH_4F + 0.1 *M* EDTA). Dissolve 55.56 g of ammonium fluoride (NH_4F) in 600 mL of deionized water in a 1 L volumetric flask. Add 29.23 g of EDTA to this mixture, dissolve, bring to 1 L volume using deionized water, mix thoroughly, and store in plastic bottle.

   b. In a 10 L plastic carboy containing 8 L of deionized water, dissolve 200.1 g of ammonium nitrate (NH_4NO_3) and add 100 mL of stock solution M3, 115 mL concentrated acetic acid (CH_3COOH), 82 mL of 10% v/v nitric acid (10 mL concentrated HNO_3 in 100 mL of deionized water), bring to 10 L with deionized water and mix thoroughly.

   c. The pH of the extracting solution should be 2.3 $\pm$ 0.2.

8 Solutions for the manual determination of phosphorus:

   a. Solution A: dissolve 12 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 250 mL of deionized water. In a 100 mL flask, dissolve 0.2908 g of potassium antimony tartrate in 80 mL of deionized water. Transfer these two solutions

into a 2 L volumetric flask containing 1000 mL of 2.5 *M* H_2SO_4 (141 mL concentrated H_2SO_4 diluted to 1 L with deionized water), bring to 2 L with deionized water, mix thoroughly, and store in the dark at 4°C.

b. Solution B: dissolve 1.056 g of ascorbic acid in 200 mL of solution A. Solution B should be fresh and prepared daily.

c. Standard solution of P: use certified P standard or prepare a solution of 100 μg mL^{-1} P by dissolving 0.4393 g of KH_2PO_4 in 1 L of deionized water. Prepare standard solutions of 0, 0.5, 1, 2, 5, and 10 μg mL^{-1} P in diluted M3 extractant.

9 Solutions for K, Ca, Mg, and Na determination by atomic absorption:

a. Lanthanum chloride ($LaCl_3$) solution: 10% (w/v).

b. Concentrated solution of cesium chloride (CsCl) and $LaCl_3$: dissolve 3.16 g of CsCl in 100 mL of the 10% $LaCl_3$ solution.

c. Combined K and Na standard solutions: use certified atomic absorption standard and prepare solutions of 0.5, 1.0, 1.5, 2.0 and 0.3, 0.6, 0.9, 1.2 μg mL^{-1} of K and Na, respectively.

d. Combined Ca and Mg standard solutions. Prepare 2, 4, 6, 8, 10 and 0.2, 0.4, 0.6, 0.8, 1.0 μg mL^{-1} of Ca and Mg, respectively.

10 Standard solution for Cu, Zn, and Mn determination by atomic absorption:

a. Combined Cu and Zn standard solution: 0, 0.2, 0.4, 0.8, 1.2 to 2.0 μg mL^{-1} of Cu and of Zn in M3 extractant.

b. Mn standard solutions: prepare 0, 0.4, 0.8, 1.2 to 4 μg mL^{-1} of Mn in diluted M3 extractant.

7.3 PROCEDURE

7.3.1 Extraction

1 Weigh 3 g of dry soil passed through a 2 mm sieve into a 125 mL Erlenmeyer flask.

2 Add 30 mL of the M3 extracting solution (soil:solution ratio 1:10).

3 Shake immediately on reciprocating shaker for 5 min (120 oscillations min^{-1}).

4 Filter through M3-rinsed Whatman #42 filter paper into plastic vials and store at 4°C until analysis.

5 Analyze elements in the filtrate as soon as possible using either an automated or manual method as described below.

7.3.2 Determination of P by Manual Colorimetric Method

1. Pipet 2 mL of the clear filtrate or standard (0 to 10 μg mL^{-1}) P solution into a 25 mL volumetric flask. The sample aliquot cannot contain more than 10 μg of P and dilution of the filtrate with M3 maybe required.

2. Add 15 mL of distilled water and 4 mL of solution B, make to volume with distilled water and mix.

3. Allow 10 min for color development, and measure the absorbance at 845 nm.

7.3.3 Determination of K, Ca, Mg, and Na by Atomic Absorption or by Flame Emission

Precipitation problems can result from the mixture of the CsCl–$LaCl_2$ solution with the M3 extract. It is therefore recommended that the extracts be diluted (at least 1:10 final dilution) as indicated below to avoid this problem.

1. Pipet 1 to 5 mL of filtrate into a 50 mL volumetric flask.

2. Add approximately 40 mL of deionized water and mix.

3. Add 1 mL of the CsCl–$LaCl_3$ solution, bring to volume with deionized water and mix.

4. Determine Ca, Mg by atomic absorption and K, Na by flame emission.

7.3.4 Determination of Cu, Zn, and Mn by Atomic Absorption

The Cu and Zn concentrations in the extract are determined without dilution while the Mn concentration is determined in diluted M3 extract.

7.3.5 Comments

1. Filter paper can be a source of contamination which may affect the end results, especially for Zn, Cu, and Na. Mehlich (1984) proposed to use 0.2% $AlCl_3$ as a rinsing solution for all labware including qualitative filter paper. Based on local tests, we suggest the use of M3 extracting solution as a rinsing solution for filter paper.

2. Because of Zn contamination, Pyrex glassware cannot be used for extraction or storage of the M3 extractant and laboratory standards.

3. Tap water is a major source of Cu and Zn contamination.

7.4 RELATIONSHIPS WITH OTHER EXTRACTANTS

The M3 extractant is widely used as ''universal extractant'' in North America, Europe, and Australia (Zbiral and Nemec 2000; Cox 2001; Bolland et al. 2003). Jones (1998) reported that M3 is becoming the method of choice since many elements can be determined with this

extractant. In Canada, it is used in the soil testing program in the provinces of Quebec and Prince Edward Island (CPVQ 1989; CRAAQ 2003). Many studies have been conducted over the world comparing the M3 method to the commonly used methods (ammonium acetate for K and DTPA for micronutrients) and report in general highly significant relationships between these methods. Some comments on relative amounts of elements extracted are provided below.

1 The amounts of K and Na extracted by M3 are equal to those determined by ammonium acetate (Tran and Giroux 1989).

2 The amounts of Ca and Mg extracted by M3 are about 1.10 times more than those extracted by ammonium acetate method (Tran and Giroux 1989).

3 The amount of Zn extracted by M3 is about one half to three quarters of the amount extracted by DTPA (Lindsay and Norvell 1978).

4 The amount of Cu extracted by M3 is about 1.8 times more than that extracted by DTPA (Makarim and Cox 1983; Tran 1989; Tran et al. 1995).

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Chapter 8
Sodium Bicarbonate-Extractable Phosphorus

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8.1 INTRODUCTION

Sodium bicarbonate ($NaHCO_3$)-extractable phosphorus, commonly termed Olsen-P (Olsen et al. 1954), has a long history of worldwide use as an index of soil-available P on which to base P fertilizer recommendations (Cox 1994). It has been successfully used as a soil test for P in both acid and calcareous soils (Kamprath and Watson 1980). As a soil test, Olsen-P is sensitive to management practices that influence bioavailable soil P levels, such as fertilizer (O'Halloran et al. 1985) or manure (Qian et al. 2004) additions, although it is not suitable for P extraction from soils amended with relatively water-insoluble P materials such as rock phosphate (Mackay et al. 1984; Menon et al. 1989).

As an extractant, $NaHCO_3$ acts through a pH and ion effect to remove solution inorganic P (P_i) plus some labile solid-phase P_i compounds such as phosphate adsorbed to free lime, slightly soluble calcium phosphate precipitates, and phosphate loosely sorbed to iron and aluminum oxides and clay minerals. Sodium bicarbonate also removes labile organic P (Bicarb-P_o) forms (Bowman and Cole 1978; Schoenau et al. 1989) that may be readily hydrolyzed to P_i forms and contribute to plant-available P (Tiessen et al. 1984; O'Halloran et al. 1985; Atia and Mallarino 2002) or be reassimilated by microorganisms (Coleman et al. 1983). Although these labile P_o fractions once mineralized may play an important role in the P nutrition of crops, most regions using the Olsen-P soil test only consider the P_i fraction. A modification of the Olsen-P method is one of the extraction steps used in the sequential extraction procedure for soil P outlined in Chapter 25. In this method, the $NaHCO_3$-extractable P_i (Bicarb-P_i) and Bicarb-P_o are determined after a 16 h extraction. If the researcher is interested in a measure of the impact of treatments or management on these labile P_i and P_o fractions, one can simply follow the $NaHCO_3$ extraction and analysis procedure outlined in Chapter 25, ignoring the initial extraction using exchange resins.

As with many soil tests for P, the Olsen-P test has been used as a surrogate measure of potential P loss through runoff (Pote et al. 1996; Turner et al. 2004) and in regions using the Olsen-P as the recommended soil P test it is often a criterion in soil P indices for assessing risk of P loss and impact on surface waters (Sharpley et al. 1994). The reader is referred to Chapter 14 for a more comprehensive discussion of methods for determining environmental soil P indices. Owing to its widespread use as an extractant for assessing P availability and its utilization in environmental P loading regulations, this chapter covers methodology for measurement of Olsen-P as a soil test.

8.2 SODIUM BICARBONATE-EXTRACTABLE INORGANIC P (OLSEN ET AL. 1954)

In this extraction, a soil sample is shaken with 0.5 *M* $NaHCO_3$ adjusted to a pH of 8.5, and the extract filtered to obtain a clear, particulate-free filtrate. The filtrate is usually a yellowish to dark brown color, depending upon the amount of organic matter removed from the soil. When relatively small amounts of organic matter are removed (pale yellowish-colored filtrates) it is possible to simply correct for its presence by using a blank correction (i.e., measure absorbance of a suitably diluted aliquot without color-developing reagent added). Presence of higher concentrations of organic matter can interfere with the color development in some colorimetric methods, or result in the precipitation of organic materials. Several options exist for the removal of the organic material in the extracts such as the use of charcoal (Olsen et al. 1954) and polyacrylamide (Banderis et al. 1976).

8.2.1 Extraction Reagents

1 Sodium bicarbonate ($NaHCO_3$) extracting solution, 0.5 *M* adjusted to pH 8.5. For each liter of extracting solution desired, dissolve 42 g of $NaHCO_3$ and 0.5 g of NaOH in 1000 mL of deionized water. The $NaHCO_3$ extracting solution should be prepared fresh each month and stored stoppered since changes in pH of solution may occur that can affect the amount of P extracted.

2 If using charcoal to remove organic material from the extracting solution: prepare by mixing 300 g of phosphate-free charcoal with 900 mL of deionized water (see Comment 2 in Section 8.2.3).

3 If using polyacrylamide to remove organic material from the extracting solution: dissolve 0.5 g of polyacrylamide in approximately 600 mL of deionized water in a 1 L volumetric flask. This may require stirring for several hours. When the polymer has dissolved, dilute to volume with distilled water.

8.2.2 Procedure

1 Weigh 2.5 g sample of air-dried (ground to pass through a 2 mm sieve) soil into a 125 mL Erlenmeyer flask. Include blank samples without soil.

2 Add 50 mL of 0.5 *M* $NaHCO_3$ extracting solution at 25°C.

3 If using charcoal to remove dissolved soil organic matter from the extracting solution: add 0.4 mL of the charcoal suspension.

4 If using polyacrylamide to remove dissolved soil organic matter from extracting solution: add 0.25 mL of the polyacrylamide solution.

5 Shake for 30 min on a reciprocating shaker at 120 strokes per minute.

6 Filter the extract into clean sample cups using medium retention filter paper (i.e., VWR 454 or Whatman No. 40). If the filtrate is cloudy, refilter as necessary.

7 See Section 8.3 for the determination of Olsen-P in the filtrates.

8.2.3 Comments

1 The conditions under which the extraction is conducted can influence the amount of P extracted from the soil. Increasing the speed and time of the shaking will usually result in greater amounts of P being extracted (Olsen and Sommers 1982). Limiting extraction times to 30 min have been adopted for most soil testing purposes although a more complete and reproducible extraction may be obtained with a 16 h extraction. Increasing temperature of extraction will also increase the amount of P extracted. Olsen et al. (1954) reported that extracted P_i increased by 0.43 mg P kg^{-1} soil for each 1°C increase in temperature between 20°C and 30°C in soils testing between 5 and 40 mg P kg^{-1} soil. It is therefore important that if the results are to be interpreted in terms of regional management recommendations, the conditions of extraction must be similar to those used for the calibration of the soil test. If the results are for a comparative purpose between samples, then uniformity of extraction conditions between sample extractions is of greater importance than selecting a specific shaking speed, duration, and temperature of extraction.

2 Most commercially available sources of charcoal or carbon black are contaminated with P. It is strongly recommended that the charcoal be washed with 6 *M* HCl to remove the P, followed by repeated washings with deionized water. Analysis of sample blanks of $NaHCO_3$ extracting solution with and without the charcoal solution will indicate if P removal from the charcoal has been successful.

3 The $NaHCO_3$ extracts should be analyzed as soon as possible, as microbial growth can proceed very rapidly, even under refrigeration. One can add one or two drops of toluene to inhibit microbial activity, although this increases the biohazard rating of the filtrates for handling and disposal. Preferably, the filtrates should be stored under refrigeration and analyzed within 5 days if they cannot be analyzed immediately.

8.3 PHOSPHORUS MEASUREMENT IN THE EXTRACT

The amount of orthophosphate in the $NaHCO_3$ extractions is usually determined colorimetrically and various methods, both manual and automated, are available. The manual

method described here is based on one of the most widely used procedures, the ammonium molybdate–antimony potassium tartrate–ascorbic acid method of Murphy and Riley (1962). This method is relatively simple and easy to use and the manual method described is adaptable to automated systems. The addition of antimony potassium tartrate eliminates the need for heating to develop the stable blue color. The phosphoantimonylmolybdenum complex formed has two absorption maxima; one at 880 nm and the other at 710 nm (Going and Eisenreich 1974). Watanabe and Olsen (1965) suggest measuring absorbance at 840 to 880 nm utilizing the greater of the two absorbance maxima, while Chapter 25 suggests using 712 nm to reduce possible interference from traces of organic matter in slightly colored extracts.

8.3.1 Reagents for P Measurement

1. Ammonium molybdate solution: dissolve 40 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 1000 mL of deionized water.

2. Ascorbic acid solution: dissolve 26.4 g of L-ascorbic acid in 500 mL of deionized water. Store under refrigeration at ~2°C. Prepare fresh if solution develops a noticeable color.

3. Antimony potassium tartrate solution: dissolve 1.454 g of antimony potassium tartrate in 500 mL of deionized water.

4. Sulfuric acid (H_2SO_4), 2.5 *M*: slowly add 278 mL concentrated H_2SO_4 to a 2 L volumetric flask containing ~1 L of deionized water. Mix and allow to cool before making to volume with distilled deionized water.

5. Sulfuric acid (H_2SO_4), ~0.25 *M*: slowly add ~14 mL concentrated H_2SO_4 to a 100 mL volumetric flask containing ~75 mL of distilled water. Mix well and make to volume with distilled water.

6. *p*-nitrophenol solution, 0.25% (w/v): dissolve 0.25 g of *p*-nitrophenol in 100 mL of distilled water.

7. Standard P stock solution: prepare 100 mL of a base P standard with concentration of 5 μg P mL^{-1}.

8. Making the Murphy–Riley color-developing solution: using the above reagents, prepare the Murphy–Riley color-developing solution in a 500 mL flask as follows: add 250 mL of 2.5 *M* H_2SO_4, followed by 75 mL of ammonium molybdate solution, 50 mL of ascorbic acid solution, and 25 mL of antimony potassium tartrate solution. Dilute to a total volume of 500 mL by adding 100 mL of deionized water and mix on a magnetic stirrer. The reagents should be added in proper order and the contents of the flask swirled after each addition. Keep the Murphy–Riley solution in an amber bottle in a dark location to protect from light. Fresh Murphy–Riley solution should be prepared daily.

8.3.2 Procedure

1. Pipette 10 mL or a suitable aliquot of the filtered $NaHCO_3$ extract into a 50 mL volumetric flask. Include both distilled water and $NaHCO_3$ blanks. (See Comment 2 in Section 8.3.3).

2. To prepare standards of desired concentration range: 0, 0.1, 0.2, 0.3, 0.4, and 0.8 μg P mL^{-1} in $NaHCO_3$ matrix, add 0, 1, 2, 3, 4, 6, and 8 mL of the base P standard (5 μg P mL^{-1}) to 50 mL volumetric flasks. Then add 10 mL of 0.5 *M* $NaHCO_3$ to each flask.

3. To adjust the pH of the solutions add one to two drops of *p*-nitrophenol to each flask, which should result in a yellow solution. Lower the pH by adding 0.25 *M* H_2SO_4 until the solution just turns colorless.

4. To each flask, add 8 mL of the Murphy and Riley color-developing solution prepared in Section 8.3.1. Make to volume (50 mL) with deionized water, shake and allow 15 min for color development.

5. Measure the absorbance of the standards and samples on a suitably calibrated and warmed-up spectrophotometer set to either 712 or 880 nm. Construct a standard curve using the absorbance values from standards of known P concentration.

8.3.3 Comments

1. The ammonium molybdate, ascorbic acid, and antimony potassium tartrate solutions are generally stable for 2 to 3 months if well stoppered and stored under refrigeration. If quality of the solutions or reagents is suspected, discard and prepare fresh, as deterioration and/or contamination is a common source of error in the analysis.

2. Although several modifications of the Murphy and Riley procedure exist in the literature, when using reagents as originally described by Murphy and Riley (1962) the final concentration of P in the 50 mL volumetric flask should not exceed 0.8 μg P mL^{-1} (Towns 1986) as color development may not be complete. Thus, the suitable aliquot size for color development should contain <40 μg P. See Chapter 24 (Section 24.5) for more discussion on color development using the Murphy and Riley reagents.

8.3.4 Calculation

Using the concentrations of P suggested in Section 8.3.2, the standard curve should be linear. If the standard curve is constructed based on the μg P contained in the 50 mL flask (i.e., 0, 5, 10, 15, 20, 30, and 40 μg P) vs. absorbance, then the sample P content in mg P kg^{-1} soil can be calculated using the following formula:

$$\text{mg P kg}^{-1}\text{ soil} = \mu\text{g P in flask} \times \frac{\text{50 mL (extraction volume)}}{\text{mL aliquot}} \times \frac{1}{\text{g of soil}} \qquad (8.1)$$

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Chapter 9
Boron, Molybdenum, and Selenium

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9.1 INTRODUCTION

Common features of B, Mo, and Se are that all three are nutrient elements that can be mainly found either in anionic or neutral form in soil solution and are relatively mobile in soils. Boron and Mo are essential elements for both plants and animals, while Se is an important element for humans and animals. Both B and Mo are essential micronutrients required for the normal growth of plants, with differences between plant species in the levels required for normal growth of plants. There is a narrow soil solution concentration range defining B or Mo deficiencies and toxicities in plants.

Boron deficiencies can be found most often in humid regions or in sandy soils. Boron is subject to loss by leaching, particularly in sandy soils, and thus responses to B are common for sandy soils as summarized by Gupta (1993). Responses to B have been found on a variety of crops in many countries (Ericksson 1979; Touchton et al. 1980; Sherrell 1983). In contrast, B toxicity can be found mostly in arid and semiarid regions either due to high B in soils or high B containing irrigation water (Keren 1996).

Responses to Mo have been frequently observed in legumes grown on soils that need lime. Elevated levels of Mo in soils and subsequent accumulations of Mo in plants, however, are of more concern than Mo deficiency in soils. High levels of Mo in plants eaten by ruminants can induce molybdenosis, a Mo-induced Cu deficiency (Jarrell et al. 1980).

Yield responses to Se are generally not found. However, it is essential for livestock and is somewhat unique among the essential nutrients provided by plants to animals. In some areas, native vegetation can contain Se levels that are toxic to animals, whereas in other locations,

vegetation can be deficient in Se, also causing animal health problems due to inclusion of low Se forage as part of animal diets (Mikkelsen et al. 1989). The Se concentration in soils in humid regions is generally inadequate to produce crops sufficient in Se to meet the needs of livestock. In acid soils, the ferric-iron selenite complex is formed, which is only slightly available to plants (NAS-NRC 1971). Selenium is generally present in excessive amounts only in semiarid and arid regions in soils derived from cretaceous shales, where it tends to form selenates (Welch et al. 1991). Selenium toxicity problems in the semiarid western United States are generally associated with alkaline soils where Se is present in the selenate form (Jump and Sabey 1989).

9.2 BORON

Boron in soils is primarily in the +3 oxidation state taking the form of the borate anion: $B(OH)_4^-$. The two most common solution species of B are neutral boric acid (H_3BO_3) and borate anion ($B(OH)_4^-$). Boron in soil can either be present in soil solution or adsorbed onto soil minerals such as clays. Below pH 7, H_3BO_3 predominates in soil solution, resulting in only a small amount of B adsorbed onto soil minerals. As the pH increases to about 9, the $B(OH)_4^-$ increases rapidly, increasing B adsorption (Vaughan and Suarez 2003). Only the B in soil solution is important for plants.

A number of extractants such as 0.05 *M* HCl (Ponnamperuma et al. 1981), 0.01 *M* $CaCl_2$ + 0.5 *M* mannitol (Cartwright et al. 1983), hot 0.02 *M* $CaCl_2$ (Parker and Gardner 1981), and 1 *M* ammonium acetate (Gupta and Stewart 1978) have been employed for determining the availability of B in soils. One advantage of using $CaCl_2$ is that it extracts little color from the soil, and predicted error due to this color is found to be low at 0.00–0.07 mg kg^{-1} (Parker and Gardner 1981). Such filtered extracts are also free of colloidal matter.

Oyinlola and Chude (2002) reported that only hot water-soluble B correlated significantly with relative yields in Savannah soils of Nigeria, compared to several other extractants. Likewise Matsi et al. (2000) in northern Greece also noted that hot water-soluble B provided better correlation with yields than ammonium bicarbonate-diethylenetriamine-pentaacetic acid (AB-DTPA). Similar results were reported on some Brazilian soils where hot water-soluble B proved to be superior to HCl and mannitol in predicting the B availability for sunflower (Silva and Ferreyra 1998). Moreover, research work by Chaudhary and Shukla (2004) on acid soils of western India showed that both 0.01 *M* $CaCl_2$ and hot water extractions were suitable for determining the B availability to mustard (*Brassica juncea*).

Contrary to most other findings, Karamanos et al. (2003) concluded that hot water-extractable B was not an effective diagnostic tool for determining the B status of western Canadian soils. They, however, stressed that soil properties, especially organic matter, played an important role in determining the fate of applied B in the soil–plant system. Raza et al. (2002), on the other hand, found hot water-soluble B to be a good estimate of available B in the prairie soils of Saskatchewan. They further stated that soil cation exchange capacity appeared to be an important characteristic in predicting the B availability.

The most commonly used method is still the hot water extraction of soils as originally developed by Berger and Truog (1939) and modified by Gupta (1993). A number of

modified versions of this procedure have since appeared. Offiah and Axley (1988) have used B-spiked hot water extraction for soils. This method is claimed to have an advantage over unspiked hot water extraction in that it removes from consideration a portion of the B fixing capacity of soils that does not relate well to plant uptake. A longer boiling time of 10 min as opposed to the normally used 5 min boiling was found to reduce error for a Typic Hapludult soil by removing enough B to reach the plateau region of the extraction curve (Odom 1980).

Once extracted from the soil, B can be analyzed by the colorimetric methods using reagents such as carmine (Hatcher and Wilcox 1950), azomethine-H (Wolf 1971), and most recently by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Keren 1996).

9.2.1 Reagents

1 Deionized water

2 Charcoal

9.2.2 Procedure (Gupta 1993)

1 Weigh 25 g air-dried soil, screened through a 2 mm sieve, into a preweighed 250 mL ''acid-washed'' beaker and add about 0.4 g charcoal and 50 mL deionized water and mix. The amount of charcoal added will vary with the organic matter content of the soil and should be in sufficient quantity to produce a colorless extract after 5 min of boiling (see Comments 2 and 3 in Section 9.2.5). A blank containing only deionized water and a similar amount of charcoal as used with the soil samples should also be included.

2 Boil the soil–water–charcoal or water–charcoal mixtures for 5 min on a hotplate.

3 The loss in weight due to boiling should be made up by adding deionized water and the mixture should be filtered while still hot through a Whatman No. 42 or equivalent type of filter paper.

9.2.3 Determination of Boron by the Azomethine-H Method

Reagents

1 Azomethine-H: dissolve 0.5 g azomethine-H in about 10 mL redistilled water with gentle heating in a water bath or under a hot water tap at about 30°C. When dissolved add 1.0 g L-ascorbic acid and mix until dissolved. Make the final volume up to 100 mL with redistilled water. If the solution is not clear, it should be reheated again till it dissolves. Prepare fresh azomethine-H solution for everyday use.

2 Ethylene diamine tetraacetic acid (EDTA) reagent (0.025 *M*): dissolve 9.3 g EDTA in redistilled water and make the volume up to 1 L with redistilled water. Add 1 mL Brij-35 and mix.

3 Buffer solution: dissolve 250 g ammonium acetate in 500 mL redistilled water. Adjust the pH to about 5.5 by slowly adding approximately 100 mL concentrated acetic acid, with constant stirring. Add 0.5 mL Brij-35 and mix.

4 Standard solutions: prepare stock standard A by dissolving 1000 mg B (5.715 g H_3BO_4) in 1 L deionized water and prepare stock standard B by taking 50 mL stock standard A and diluting it to 1 L with 0.4 *M* HCl. Prepare standard solutions from stock standard B by diluting a range of 2.5 to 30 mL stock standard B to 1 L with deionized water to give a range of 0.5 to 6.0 mg B L^{-1} in the final standard solution.

Procedure

1 Take 5 mL of the clear filtrate in a test tube and add 2 mL buffer solution, 2 mL EDTA solution, and 2 mL azomethine-H solution, mixing the contents of the test tube thoroughly after the addition of each solution.

2 Let the solutions stand for 1 h and measure the absorbance at 430 nm on a spectrophotometer.

3 The color thus developed has been found to be stable for up to 3–4 h.

4 The pH of the colored extract should be about 5.0.

9.2.4 Determination of Boron by Inductively Coupled Plasma-Atomic Emission Spectrometry

This technique has been found to be rapid and reliable for determining B in plant digests and soil extracts using the procedure described in Section 9.2.2 by Gupta (1993). An estimated detection limit by ICP-AES at wavelength of 249.77 nm is about 5 μg L^{-1} (APHA 1992) and therefore, it is reasonable to expect method detection limit to be about 100 μg B kg^{-1} soil. Care must be taken to filter samples properly as colloidal-free extracts are recommended for ICP-AES to avoid nebulizer-clogging problems.

9.2.5 Comments

1 All glassware used in plant or soil B analyses must be washed with a 1:1 mixture of boiling HCl acid with deionized water before use. Storage of the filtered extracts before the analysis of B must be in plastic sampling cups.

2 Soils containing higher organic matter may require additional amount of charcoal to obtain a colorless extract, but the addition of excessive amounts of charcoal can reduce the amount of B in the extract.

3 If the filtered solution is not colorless, the extraction may need to be repeated with a higher amount of charcoal.

4 The use of azomethine-H is an improvement over those of carmine (Hatcher and Wilcox 1950), quinalizarin, and curcumin (Johnson and Ulrich 1959), since the

procedure involving this chemical does not require use of a concentrated acid. This method has been found to give comparable results when compared to the carmine method (Gupta 1993).

5 It is difficult to use an autoanalyzer because of its insensitivity at lower B concentrations generally found in the hot water extract of most soils.

9.3 MOLYBDENUM

Molybdenum in soils is primarily in the +6 oxidation state taking the form of the molybdate anion, MoO_4^{2-}. The solution species of Mo, generally in the order of decrease in concentration, are MoO_4^{2-}, $HMoO_4^{-}$, $H_2MoO_4^{0}$, $MoO_2(OH)^{+}$, and MoO_2^{2+}, respectively. The latter two species can be ignored in most soils (Lindsay 1979). Molybdate is adsorbed by oxides, noncrystalline aluminosilicates, and to a lesser extent by layer silicates and adsorption increases with decreasing pH. Therefore, Mo is least soluble in acid soils, especially acid soils containing Fe oxides.

Studies on the extraction of available Mo from soils have been limited. Further, the extremely low amounts of available Mo in soils under deficiency conditions make it difficult to determine Mo accurately. The accumulation of Mo in plants mostly is not related to total concentrations of Mo in soils but rather to available Mo in soils. A variety of extractants have been used in attempts to extract available Mo in soils although no routine soil test for Mo is available. Molybdenum deficiencies are rare and are mostly of concern for leguminous crops. Since excessive Mo in forages can harm animal health, Mo fertilization is usually based on visual deficiency symptoms and/or history of crop rotation.

Many extractants have been employed for the assessment of available Mo in soils. Those extractants are: ammonium oxalate, pH 3.3 (Grigg 1953); water (Gupta and MacKay 1965a); hot water, anion-exchange resin; AB-DTPA (Soltanpour and Workman 1980); ammonium carbonate (Vlek and Lindsay 1977); and Fe oxide strips (Sarkar and O'Connor 2001). However, most of those extractants are used to study the deficiency aspect rather than from consideration of toxic effects (Davies 1980).

Despite its weaknesses, the most commonly used extractant for assessing Mo availability in soils has been ammonium oxalate, buffered at pH 3.3 (Grigg 1953). Examples for the successful use of acid ammonium oxalate in predicting Mo uptake by plants (Wang et al. 1994) and its failures (Mortvedt and Anderson 1982; Liu et al. 1996) can be found in the literature. From studies that failed to predict plant uptake of Mo successfully with acid ammonium oxalate-extractable Mo, it appeared that plant Mo was more closely related to some soil property such as pH other than extractable Mo in soils. Some studies obtained a better regression between acid oxalate-extractable Mo in soil and plant Mo when soil pH was considered as a factor (Mortvedt and Anderson 1982). Sharma and Chatterjee (1997) stated that soil physical properties such as soil pH, organic matter, parent rock, and texture play an important role in determining the Mo availability in alkaline soils. Multiple-regression equations account for the contribution of the individual factors, which would make the critical limits more predictable. Moreover, Liu et al. (1996) found significant correlations ($r^2 = 0.81$) for soil Mo extracted with ammonium oxalate (pH 6.0) in a group of Kentucky soils with Mo uptake by tobacco (*Nicotiana tabacom* L.) growing in

greenhouse. However, ammonium oxalate buffered at pH 3.3 was not statistically well correlated with Mo uptake.

Some methods that have not been widely tested but appear to be promising are anion-exchange resin and AB-DTPA methods. Anion-exchange resins have been used with success to extract plant-available Mo in soils (Ritchie 1988). The AB-DTPA method (Soltanpour and Workman 1980; Soltanpour et al. 1982) has also been used successfully for alkaline and Mo-contaminated soils (Pierzynski and Jacobs 1986, Wang et al. 1994). Moreover, ammonium carbonate (Vlek and Lindsay 1977) also has shown good correlation with plant uptake of Mo, especially for soils that have Mo toxicity problems. This extraction followed by H_2O_2 treatment leaves a decolorized extract that is useful for Mo analysis by colorimetric methods (Wang et al. 1994).

To characterize the available Mo in biosolids-amended soils, Sarkar and O'Connor (2001) compared the potential of Fe-oxide impregnated filter paper with ammonium oxalate extraction method and total soil Mo. Their data showed that the best correlation between plant Mo and soil Mo was obtained using the Fe-oxide strip followed by ammonium oxalate extraction; while total soil Mo was generally not well correlated with plant Mo uptake. Sarkar and O'Connor (2001) further reported that Fe-oxide strips can serve as an analytically satisfactory and practical procedure for assessing available Mo, even in soils amended with biosolids.

Recently, McBride et al. (2003) found that dilute $CaCl_2$ was found to be preferable to Mehlich 3 as a universal extractant for determining Mo and other trace metal availability in clover grown on near neutral soils amended with sewage sludge. Concentration of Mo in alfalfa (*Medicago sativa* L.) on soils treated with sewage sludge was well correlated to readily extractable Mo by 0.01 *M* $CaCl_2$ in the soil. Total Mo and past Mo loading to soil were less reliable predictors of Mo concentration in alfalfa than the soil test for readily extractable Mo (McBride and Hale 2004).

Two methods of extractions are outlined (1) ammonium oxalate, pH 3.0 (modified Grigg 1953) and (2) AB-DTPA (Soltanpour and Schwab 1977).

9.3.1 Extraction of Molybdenum by the Ammonium Oxalate, pH 3.0 Method (Modified Grigg 1953)

Reagents (Gupta and MacKay 1966)

1 Ammonium oxalate, 0.2 *M* buffered to pH 3.0: in a 1 L volumetric flask dissolve 24.9 g of ammonium oxalate and 12.605 g of oxalic acid in approximately 800 mL deionized water. Make to volume with distilled water and mix well.

Procedure

1 Add 15 g air-dried soil, screened through a 2 mm sieve, to a 250 mL beaker or Erlenmeyer flask.

2 Add 150 mL of the buffered (pH 3) 0.2 *M* ammonium oxalate solution and shake for 16 h at room temperature using an orbital shaker at 200 rpm.

3 Filter the extraction through Whatman No. 42 filter paper or equivalent. Centrifuge the filtrate for 20 min.

4 Determine Mo concentration in the clear extract as described in Section 9.3.3. If required, the centrifuged extracts can be acidified to pH < 2 with HNO_3 and stored in 1:1 HNO_3 rinsed plastic or glass containers up to a maximum of 6 months (APHA 1992).

9.3.2 Extraction of Molybdenum by the Ammonium Bicarbonate-Diethylenetriaminepentaacetic Acid Solution Method (Soltanpour and Schwab 1977)

Reagents

1 Ammonium hydroxide (NH_4OH) 1:1 solution.

2 AB-DTPA solution (1 *M* NH_4HCO_3, 0.005 *M* DTPA buffered to pH 7.6): in a 1 L volumetric flask containing approximately 800 mL of distilled-deionized water, add 1.97 g of DTPA and approximately 2 mL of 1:1 NH_4OH solution and mix. (The addition of the 1:1 NH_4OH solution aids in the dissolution of DTPA and helps prevent frothing.) When most of the DTPA is dissolved, add 79.06 g of NH_4HCO_3 and stir until all materials have dissolved. Adjust pH to 7.6 by adding either NH_4OH or HCl and then make to volume using distilled-deionized water.

Procedure

1 Weigh 10 g soil, screened through a 2 mm sieve, into a 125 mL Erlenmeyer flask and add 20 mL of AB-DTPA solution.

2 Shake the mixture in open flasks on a reciprocal shaker at 180 rpm for 15 min and filter the extract using Whatman No. 42 filter paper or its equivalent.

3 Determine Mo as described in Section 9.3.3. The filtered extracts can be preserved until analysis as mentioned under Section 9.3.1 (Reagents (1)).

9.3.3 Determination of Molybdenum

Determine Mo concentration in extracts with graphite furnace atomic absorption spectrometry (GFAAS) or ICP-AES. The standards for GFAAS or ICP must be prepared in the extracting solution matrix.

Since extractable Mo in normal situations is usually in the range of 10 to 50 $\mu g\ L^{-1}$, analytical methods must be sensitive to measure low concentrations. Therefore, most suitable method is GFAAS (Mortvedt and Anderson 1982). It is recommended to use HNO_3 as a matrix modifier (as enhancer); and pyrolytically coated tubes (to minimize problems due to carbide formation) for Mo determination in GFAAS. An estimated detection

limit using pyrolytic graphite tubes is 1 μg L^{-1} (APHA 1992). In situations where one could expect higher concentrations of Mo in the extracting solutions, flame atomic absorption spectrometry or atomic emission spectrometry (either direct or ICP-AES) can be used for Mo analysis (Soltanpour et al. 1996). An estimated detection limit using ICP-AES is 8 μg L^{-1} (APHA 1992) and therefore, it will be safer to assume method detection limits for ICP-AES for Mo to be 80 μg L^{-1} or little lower. For spectrometry determinations standards must be made in AB-DTPA matrix solution. It has also been suggested to treat the extract with concentrated HNO_3 acid before determination of Mo by ICP-AES. After adding 0.5 mL concentrated HNO_3 acid to about 5 mL filtrate, mix it in a beaker on a rotary shaker for about 15 min to eliminate carbonate species.

Determination of Mo in soil extracts can also be done colorimetrically in laboratories that are not equipped with ICP-AES or GFAAS. Refer to Gupta and MacKay (1965b) for details of colorimetric determination of Mo.

9.3.4 Comments

In general, ammonium oxalate shows greater ability to extract Mo from soils and mine spoils compared to AB-DTPA method (Wang et al. 1994).

9.4 SELENIUM

Soil Se forms include very insoluble reduced forms including selenium sulfides, elemental Se (Se^0), and selenides (Se^{-2}) and more soluble selenate (SeO_4^{2-}), and selenite ($HSeO_3^-$, SeO_3^{2-}). Elemental Se, sulfides, and selenides only occur in reducing environments. They are insoluble and not available for plants and living organisms (McNeal and Balistrieri 1989). In alkaline, oxidized soils, selenates are the dominant forms while in slightly acidic, oxidized soils, selenites are dominant. Selenate and selenite precipitates and minerals are highly soluble in aerobic environments and therefore, the solubility of Se is controlled mainly by adsorption and complexation processes. Selenite is proven to be strongly adsorbed to soil surfaces while selenate is weakly adsorbed (Neal et al. 1987).

The parent material has a significant effect upon the Se concentration in plants. For example, field studies conducted on wheat in west central Saskatchewan showed higher Se values in wheat plants grown on lacustrine clay and glacial till, intermediate in plants grown on lacustrine silt, and lowest on aeolian sand (Doyle and Fletcher 1977). A similar trend characterized the C horizon soil, with highest Se values associated with lacustrine clay and lowest with aeolian sand. The findings of Doyle and Fletcher (1977) pointed to the potential usefulness of information on the Se content of soil parent materials when designing sampling programs for investigating regional variations in plant Se content.

Available Se in soils is highly variable. Although there were instances where a direct correlation between soil Se content and the plant grown on those soils existed (Varo et al. 1988), more often the total Se in soil proved to be of little value in predicting plant uptake (Diaz-Alarcon et al. 1996). Selenium uptake by plants depends not only on the form and partitioning of Se species between solution and solid phases but also on the presence of other ions in soil solution (such as SO_4^{-2}) and the species of plants (Bisbjerg and Gissel-Nielsen 1969; Mikkelsen et al. 1989). Therefore, ideally extractants capable of predicting or evaluating plant-available Se should be capable of extracting Se in soil solution as well as Se associated with solid phases that would be potentially released into soil solution. The ability

of an extractant to correlate significantly with plant uptake could vary depending on many factors, some of which are soil type, plant species, season, and location. Uptake of Se by plants and methods that can be used to predict and evaluate plant uptake of Se can be found in the literature (Soltanpour and Workman 1980; Soltanpour et al. 1982; Jump and Sabey 1989; Mikkelsen et al. 1989).

Soltanpour and Workman (1980) found a high degree of correlation between extracted Se by an AB-DTPA extraction procedure developed by Soltanpour and Schwab (1977) and Se uptake by alfalfa for five levels of Se(VI) in a greenhouse study. In addition, they found very high ($r^2 = 0.99$) correlation between AB-DTPA extractable and hot water-extractable Se (Black et al. 1965). The hot water-extractable Se soil test method is developed based on the assumption that soil and soil-like materials that contain appreciable amounts of water-soluble Se (majority as selenates) will give rise to Se-toxic vegetation (Black et al. 1965). Similarly, AB-DTPA should extract water-soluble Se as well as exchangeable selenate and/or selenite into solution due to bicarbonate anion. In addition, Soltanpour et al. (1982) found that the AB-DTPA-extractable Se in soil samples taken from a 0 to 90 cm depth in the autumn before seeding winter wheat (*Triticum aestivum* L.) correlated well with Se in grain samples ($r^2 = 0.82$) that were taken in the following summer.

Selenium in saturated paste extracts could also provide useful information about plant-available Se in soils (U.S. Salinity Laboratory Staff 1954) as mostly the soil:water ratio in these pastes can be related to field soil water content in a predictable manner. Using two Se-accumulating plant species, Jump and Sabey (1989) found that Se in saturated paste water extracts correlated highest with plant Se concentrations from a study that compared Se extracted from 18 different soils and mine-spoil materials by several different extractants (AB-DTPA, DTPA, hot water, saturated paste extract, and Na_2CO_3).

In addition to measuring total extractable Se, determination of Se species in soil solution, saturate paste extract, or any other extraction may also provide insight into potential for plant Se uptake. Mikkelsen et al. (1989) discussed the different mechanisms associated with energy-dependent uptake of Se(VI) and energy-independent uptake of Se(IV). They also discussed the variable uptake of Se by different plant species, which is an additional complication. Davis (1972a,b) demonstrated the variability for absorbing Se among different species within a single plant genus in two greenhouse experiments. All the above suggest that speciation information on Se(VI) and Se(IV) in extractions or soil solutions may also provide useful information on uptake of Se by plants.

Relatively labile forms of Se in soils can be evaluated by using orthophosphate (PO_4) as a soil extractant (Fujii et al. 1988). This is based on the assumptions that PO_4 replaces adsorbed forms of Se and the dominant adsorbed species of Se in these soils is Se(IV). Fujii and Burau (1989) used 0.1 *M* PO_4 solution adjusted to pH 8 and was able to extract 89% to 103% of the sorbed Se(IV) for three surface soils.

Sequential extraction procedures can also be used to identify fractions of Se in soils (Chao and Sanzolone 1989; Lipton 1991) and may be related to plant uptake. The sequential extraction method developed by Chao and Sanzolone (1989) fractionates soil Se into five operationally defined fractions (soluble, exchangeable, oxide bound, sulphide/organic matter bound, and residual or siliceous material associated), whereas the Lipton (1991) method fractionates soil Se into nine operationally defined fractions (soluble, ligand exchangeable, carbonates, oxidizable, easily reducible oxides bound, amorphous oxide bound, crystalline oxide bound, alkali-soluble Al/Si bound, and residual).

9.4.1 Extraction of Selenium in Soils

We will outline five commonly used methods of extractions with appropriate references here.

Five commonly used extractants for Se are given below:

1. AB-DPTA (Soltanpour and Schwab 1977): 10 g of air-dried soil, screened through a 2 mm sieve, is placed in a 125 mL Erlenmeyer flask. Add 20 mL of 1 *M* NH_4HCO_3 + 0.005 *M* DTPA (prepared as described in Section Reagents, p. 101) at pH 7.6. Shake the mixture in an open flask on a reciprocal shaker at 180 rpm for 15 min and filter the extract using Whatman No. 42 filter paper or its equivalent.

2. Hot water (Black et al. 1965): place 10 g of air-dried soil, sieved through a 2 mm sieve, in a 250 mL Erlenmeyer flask. Add 50 mL distilled water, and reflux over a boiling water bath for 30 min. Filter the soil suspension using Whatman No. 42 filter paper or its equivalent.

3. Saturated paste extractants (U.S. Salinity Laboratory Staff 1954): weigh 200 to 400 g of air-dried soil, sieved through a 2 mm sieve into a plastic container with a lid. Weigh the container, and container plus soil. Add distilled water to the soil, while stirring, until soil is nearly saturated. Cover the container and allow the mixture to stand for several hours. Then add more water with stirring to achieve a uniformly saturated soil–water paste. The criteria for saturation should be checked as given here (soil paste glistens as it reflects light, flows slightly when the container is tipped, slides freely and cleanly off a smooth spatula, and consolidates easily by tapping or jarring the container after a trench is formed in the paste with the side of the spatula). Allow the sample to stand for another 2 h, preferably overnight, and then recheck for the sample for saturation criteria. If the paste is too wet, add known amount of dry soil to the paste. Once saturation is attained, weigh the container plus content to get the amount of water added. Transfer the paste to a Büchner funnel fitted with highly retentive filter paper, and apply a vacuum to collect saturation extract in a test tube.

4. 0.005 *M* DTPA, 0.01 *M* $CaCl_2$ (2 h DTPA test) (Lindsay and Norvell 1978): 10 g air-dried soil, screened through a 2 mm sieve, is placed in a 50 mL polypropylene centrifuge tube. Add 20 mL of 0.005 *M* DTPA, 0.01 *M* $CaCl_2$ buffered at pH 7.3 with triethanolamine and shake for 2 h on a reciprocating shaker. Centrifuge immediately at 3000 g and filter the supernatant using Whatman No. 42 filter paper or its equivalent.

5. 0.5 *M* Na_2CO_3 (Jump and Sabey 1989): 5 g of air-dried soil, screened through a 2 mm sieve, is shaken on a reciprocating shaker in 20 mL of 0.5 *M* Na_2CO_3 solution at pH 11.3 for 30 min. Filter the extract using Whatman No. 42 filter paper or its equivalent.

Procedure

1. The soil:extractant ratio varies from 1:2 to 1:5 and the extraction time from 15 min to 2 h as given in the above-mentioned references or as summarized by Jump and Sabey (1989).

2 The filtered extracts can be analyzed for Se using a hydride-generating system attached to an ICP-AES (Soltanpour et al. 1996). Filtered extracts to be analyzed for Se can be preserved until analysis with either HNO_3 or HCl (pH < 2) to prevent loss of Se from solution (through coprecipitation or methylation of Se followed by volatilization).

All of the above five extractants when tested on soils containing high Se showed high correlation between wheat plant Se and Se extracted from soils (Jump and Sabey 1989). However, Se extracted in saturated soil pastes and expressed as mg Se L^{-1} of extract was found to be the best predictor of Se uptake in Se-accumulating plants. Furthermore, the results suggest that soil or mine-spoil materials that yield more than 0.1 mg Se L^{-1} in saturated extract may produce Se-toxic plants.

In addition, the AB-DTPA extract has been found to predict Se availability better when Se in wheat grain was correlated with Se in the 0–90 cm depth of soil as opposed to the 0–30 cm depth (Soltanpour et al. 1982). This was found to be particularly useful to screen soils and overburden material for potential toxicity of Se.

9.4.2 Determination of Selenium

Selenium in extracting solutions can be accurately determined by hydride generation atomic absorption spectrometry (HGAAS), electrothermal, or GFAAS, ICP-AES as well as combination of chemical methods with colorimetry and fluorometry (APHA 1992). The most common method of choice is the continuous HGAAS. For determination of Se at higher concentration, the ICP-AES coupled with HG may be preferred, in particular when simultaneous determination of other elements such as As is required (Workman and Soltanpour 1980). Matrix matching techniques (for example prepare standards in the same matrix as soil extracts) and extensive QA/QC procedures should be used to assure the quality of determination. For detailed information regarding the HGAAS apparatus and reagents needed for determination of Se, refer to APHA (1992) and Huang and Fujii (1996).

9.4.3 Comments

1 The extractants developed have been found to be suitable for predicting the availability of Se in Se toxic areas only. Because of rather small quantities of available Se in Se-deficient areas, no reliable extractant has yet been developed for such soils. Therefore, plant Se and total soil Se will continue to serve as the best tools available for testing the Se status of Se-deficient soils.

2 The term deficiency or deficient in connection with Se has implications in livestock and human nutrition only and not in plant nutrition since no known yield responses to Se have been found on cultivated crops.

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Chapter 10
Trace Element Assessment

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10.1 INTRODUCTION

The current literature contains a wide range of extractants that have been used to evaluate different fractions of metals and metalloids in soils (Tessier et al. 1979; Ross 1994; Ure 1996; Mihaljevic et al. 2003). These techniques fall into two categories: single or sequential extractions. Although sequential extractions have gained considerable popularity, they do have several drawbacks (Beckett 1989; Lo and Yang 1998; Shiowatana et al. 2001). From an analytical point of view, sequential extractions may result in inconsistent results due to reprecipitation of the elements of interest from one extractant to the next and errors caused by adding the different fractions can lead to values that do not agree with analyses of total metals. Perhaps the most important criticism of sequential extractions is that they are not really specific for the intended fraction; examples of extractions that do not remove specific and identifiable chemical forms are abundant in the literature (Beckett 1989; Mihaljevic et al. 2003).

The approach taken here is to select a series of single extractants that range from weak to very strong. Each of the extractants proposed in this chapter selectively dissolves some portion of the total element pool in the soil but no attempt is made to relate this to a specific type of surface or material. For our purposes it is not really important where the trace elements are held on the soil; it is more important that the analysis provides a means of predicting or explaining the interactions of the elements with biota or mobility in the soil system. In some cases, there is extensive literature that can help to relate the results to bioavailability of the elements to specific organisms.

Some of the metals such as Cd, Cu, Ni, Pb, and Zn have received considerable attention over the last 10 years. For these metals there are numerous references that relate the amounts of metals extracted by different chemicals to a biological effect (toxicity or uptake). However, other metals and metalloids are also significant contaminants in soils affected by

anthropogenic activity, but these metals have received much less attention (As, Co, Cr, Mo, Sb, Se, Tl, etc.). In these cases the number of references relating selective chemical extraction results to biological effects is much less abundant but has been growing in recent years (De Gregori et al. 2004). Although in some cases the methods proposed below have not been tested for a wide range of metals and metalloids, by providing a series of standard tests we hope that more studies will be conducted so that a database of response data can be developed. Since the elements forming oxyanions, such as As, Cr, Mo, and Se, behave quite differently in soils compared to the cationic metals, some authors prefer to use extraction procedures developed for phosphate (Van Herreweghe et al. 2003). However, many of the techniques used for the extraction of the metalloid As, for example, were originally developed for cationic metals but yield good results nonetheless (Hall et al. 1996; Mihaljevic et al. 2003).

Four extraction procedures are proposed here and are presented in the order of increasing strength:

1 A column leaching method using water and 80 μM $CaCl_2/CaSO_4$ solution

2 A weak salt solution using 0.01 M $CaCl_2$

3 A strong chelating agent (0.05 M NH_4-ethylenediaminetetraacetic acid [EDTA] partially neutralized with NH_4^+)

4 A strong acid microwave digestion procedure using HNO_3 (USEPA method 3051)

Recent work in our laboratory has led to the development of a column leaching technique that provides a very good simulation of the solubility of trace elements, pH, and ionic strength of solutions collected in the field from forest soils in Ontario and Quebec, Canada (MacDonald et al. 2004a,b). This method consists of an initial washing of the soil with deionized water, followed by an equilibration with very dilute (80 μM) $CaCl_2$ and $CaSO_4$ solution to simulate the ionic strength observed in forest soils. It has been chosen because it provides the extraction procedure best suited to estimate metal mobility under field conditions.

The $CaCl_2$ method is gaining support in Europe and North America as one of the best ways of evaluating bioavailability chemically (Houba et al. 1996; Ure 1996; Peijnenburg et al. 1999; McBride et al. 2003; Walker et al. 2003; Bongers et al. 2004). The method has the advantage of being simple to use in the laboratory and the results between laboratories are less variable than with some other methods (Quevauviller 1998). This is the same solution as is used to measure soil pH in many laboratories. A similar solution but with a slightly higher concentration is also recommended in Chapter 11 for use in estimating bioavailable Al and Mn. Gray et al. (2003) compared several extraction procedures to the ''labile pool'' as measured by isotope dilution; they found that $CaCl_2$ provided the closest comparison to this pool.

It is well known that metal and metalloids added to soils may become strongly bound to the soil particle surfaces (Ross 1994). Whether this is due to specific adsorption or precipitation, the elements that become fixed are mostly found on sites that are in contact with the soil solution. A strong chelating agent should be able to remove trace elements from a wide range of surface adsorption/precipitation sites. Although all of this ''fixed'' metal would not be immediately available, there are studies that show a good correlation between

EDTA-extractable metal and content in biological tissue (Ure 1996; De Gregori et al. 2004). The extraction with 0.05 *M* EDTA is a good choice for estimating this ''potentially available'' fraction (Quevauviller 1998).

The choice of digestion methods is wide and the USEPA alone recommends four different acid mixtures or procedures (Ming and Ma 1998). Total metal content is only obtained when HF is included in the digestion procedure; otherwise silicate minerals are not dissolved. Most laboratories prefer to use a method that does not include HF due to the danger of working with it; HF causes severe burns to skin or eyes. Trace elements found in the silicates are certainly not immediately available and there is a good chance that these trace elements are related to minerals found in the parent material rather than added by anthropogenic activity. For general laboratory purposes the HNO_3 procedure proposed here should provide a very good estimate of trace elements in contaminated soils. Although there are several alternate methods using HNO_3/HCl available (USEPA 1994), an acid mixture without HCl is preferred for inductively coupled plasma-mass spectrometry (ICP-MS) analysis. A preliminary study prepared by Canada's National Water Research Institute shows very good results with this USEPA 3051 method (Alkema and Blum 2001). It is preferred as an appropriate method for numerous elements.

10.2 COLUMN LEACHING WITH ARTIFICIAL SOIL SOLUTION (MACDONALD ET AL. 2004a,b)

Soil samples collected in the field and brought back to the laboratory for extraction yield solutions with significantly higher concentrations than solutions collected by lysimeters in the field from the same soil horizon. To obtain soil solutions that are comparable to those sampled with lysimeters it is necessary to first remove the relatively soluble material that accumulates in a sample following disturbance; this is particularly important here as we are using air-dried soil samples.

The removal of soluble material is done by ''washing'' the column with deionized water until the ionic strength drops to values similar to those found in the field. The column is then equilibrated with an artificial soil solution containing Ca, Cl, and SO_4, the ions most common in the soil solutions we have sampled in eastern Canada.

Initially researchers should monitor changes in pH and electrical conductivity during the washing and equilibrium phases so that they can see whether the concentrations are tending toward relatively constant values. The procedure described below appears to be suitable for a wide range of soils we have tested, but may not work with all soils.

The suction needed to pull the solution through the soil columns can be generated using two very different types of apparatus. The method described below uses a commercially available column extraction apparatus, although it is necessary to replace the original syringes that have a black rubber seal with ones that have an all polyethylene plunger; the leaching can also be done using a multichannel peristaltic pump but it is more difficult to achieve uniform flow rates.

10.2.1 Materials and Reagents

1 80 mL of 80 μ*M* $CaCl_2$–$CaSO_4$ for each column for the four leaching days. This is prepared by dissolving 0.0118 g of $CaCl_2 \cdot 2H_2O$ plus 0.0109 g of $CaSO_4$ in 2 L of ultrapure water.

2 Polyethylene syringes (60 mL) (HNO_3 washed) and high-density polyethylene (HDPE) frits that fit the syringes tightly.

3 Vacuum extractor capable of flow rates of 30 mL h^{-1} and 2–3 mL h^{-1}.

4 10% nitric acid (HNO_3)—trace metal grade: dilute 10 mL of concentrated acid to 100 mL in a volumetric flask.

5 Ultrapure water—usually produced by passing deionized or reverse osmosis water through a special system to produce water with an electrical conductivity less than 18 μS cm^{-1}.

6 0.45 μm membrane filters (nylon or polycarbonate).

10.2.2 Procedure

Pretreatment

1 Extractions should be carried out in an incubator at a temperature between 4°C and 6°C. The apparatus for each soil sample consists of three 60 mL syringes that are connected together vertically; only the syringe barrels are used for the upper two. The upper syringe is used as the reservoir for the solution and is connected to the middle syringe, holding the soil, with a tight-fitting stopper. The lowest syringe is slowly withdrawn by the vacuum extraction device and the solution is sucked out of the upper syringe, through the soil sample and into the bottom syringe. Make sure that there are no leaks in the system or the flow rate will be compromised.

2 Air dry, homogenize, and sieve the soils to 2 mm. Weigh and pack 15 g of mineral soil (5 g of forest floor) into a 60 mL syringe. Encase the soil between two HDPE frits. Insert the upper syringe into the column to hold the solution.

3 Add 30 mL aliquots of ultrapure water. Apply suction at a rate of 30 mL h^{-1}. After all the water has passed through the column wait 2 h before starting the next leaching. Repeat twice more for a total of 90 mL.

4 At the end of step 3, wait 2 h before starting the treatment with $CaCl_2$–$CaSO_4$.

Treatment

1 Leach the columns with 20 mL of 80 μ*M* of $CaCl_2$–$CaSO_4$ at a rate of 2 to 3 mL h^{-1} every 24 h during 4 days. Make sure that air enters the column at the end of each leaching cycle to prevent the columns from becoming anaerobic. Collect the leachates in separate acid-washed bottles.

2 Measure the pH and EC of each of the leachates. Keep the leachates from the last 3 days and mix them together to obtain one sample of about 50 mL. Filter solutions through 0.45 μm membrane filters under vacuum, and collect solutions in polyethylene bottles. Preserve the solution or a subsample of the solution (if part of the solution is being kept for other analyses) after filtration by adding 0.2 mL of 10% HNO_3 per 10 mL of solution, and analyze as soon as possible.

10.2.3 Calculations

The extraction method is not intended as a quantitative analysis of, for example, the water-soluble fraction; however it is appropriate for estimating solid-solution trace element partitioning or to estimate the concentration of trace metals in water leaching from a site.

Partitioning coefficients (K_d) are calculated as the ratio of total metals (determined through hot acid extraction, see Section 10.5) in mg kg^{-1} over metals in solution as mg L^{-1} and have the unit L kg^{-1}:

$$K_d = \frac{\text{Total metal}}{\text{Dissolved metal}} \tag{10.1}$$

10.2.4 Comments

1. Care must be taken to assure that all plasticware in contact with final solutions has been soaked 24 h in 15% HNO_3 and rinsed thoroughly with high-quality deionized water. Blanks should be carried through the entire extraction procedure to assure that solutions are not contaminated by outside sources.

2. Column methods are prone to variability. Great care must be taken to pack columns consistently. We propose adding soil in three steps and compacting the column with 10 light taps of a syringe plunger with the seal removed at each step.

3. Work in duplicate, and include blanks and quality control samples in each batch.

4. This is a fairly time-consuming procedure that takes 5 days to complete. On the first day (usually Monday), the three washing solutions are passed through the columns and collected—3 h each washing (1 h to draw the solution through and 2 h of equilibration); this makes for a 10 h day. The first $CaCl_2$–SO_4 solution is added to the columns when we leave in the evening of the first day, drawn through the columns during the night, and then collected the next morning. This leaching with the $CaCl_2$–SO_4 solution is repeated on the evenings of days 2–4.

10.3 Extraction with 0.01 *M* $CaCl_2$ (Quevauviller 1998)

10.3.1 Materials and Reagents

1. Centrifuge and 50 mL Boston-type polyethylene centrifuge tubes (HNO_3 acid washed).

2. End-over-end shaker (15 rpm).

3. Calcium chloride, 0.01 *M*; in a 1 L polyethylene volumetric flask, dissolve 1.47 g of $CaCl_2 \cdot 2H_2O$ in ultrapure water and make to volume.

4 10% nitric acid (HNO_3)—trace metal grade: dilute 10 mL of concentrated acid to 100 mL in a volumetric flask.

5 0.45 μm membrane filters (nylon or polycarbonate).

10.3.2 Procedure

1 Work at room temperature. Before taking a subsample, make sure your sample is very well homogenized by mixing the sample thoroughly for about a minute. Work in triplicates. Take a subsample of each soil to estimate moisture content. Include two blank solutions (tube and solution without soil) and two quality control samples in each batch of extractions.

2 Weigh about 2.500 g of soil into a 50 mL centrifuge tube and record weight. Add 25 mL of 0.01 *M* $CaCl_2$ to each tube, cap and shake on the end-over-end shaker for 3 h at 15 rpm.

3 Take a subsample to measure pH and discard (one per triplicate). Centrifuge at 5000 *g* for 10 min. Filter, with great care to avoid contamination, through 0.45 μm membrane under low vacuum. Keep the filtrate in a 30 mL polyethylene bottle. Preserve the solution or a subsample of the solution (if part of the solution is being kept for other analyses) after filtration by adding 0.2 mL of 10% HNO_3 per 10 mL of solution, and analyze as soon as possible. If dilutions are required, the amount of HNO_3 should be kept constant.

10.3.3 Calculations

$$\text{M } (\mu\text{g g}^{-1}) = \text{C } (\mu\text{g L}^{-1}) \times 0.025 \text{ L}/(\text{wt. soil g} \times (1 - \text{mc})) \qquad (10.2)$$

where M is the metal content, C is the concentration measured, and mc is the moisture content expressed as a 2-decimal fraction (i.e., 5% = 0.05).

10.3.4 Comments

1 Great care must be taken to avoid contamination. Polyethylene should be used to avoid sorption/desorption of metals to or from the walls of the containers. Centrifuge bottles, sample bottles, filtration units must be clean and acid washed followed by an acid soaking in 15% HNO_3 for 24 h and thoroughly rinsed with double-deionized water with a final rinse with ultrapure (or equivalent) water.

2 The version given here is an adaptation from Quevauviller's method; it is a compromise using smaller sample size for routine analysis. The reader is invited to read the original reference cited.

3 Reproducibility is difficult to achieve in this kind of extraction; care should be given to each step of the procedure.

4 As part of the quality control procedure, the analysis of one sample should be repeated in each batch of extractions to evaluate the reproducibly of the whole experiment. When the value of the quality control sample falls outside 2 standard deviations, calculated for all measurements of that sample, the whole batch should be reanalyzed.

10.4 EXTRACTION OF TRACE ELEMENTS WITH 0.05 *M* EDTA

10.4.1 Materials and Reagents

1 Centrifuge and 50 mL Boston-type polyethylene centrifuge tubes (in addition to the acid-washing procedures described in the comments, the labware must be rinsed with EDTA followed by a thorough water rinse before use in this experiment).

2 End-over-end shaker (15 rpm).

3 NH_4-EDTA salt solution 0.05 *M*: EDTA in its ammonium salt form is difficult to obtain in a pure form. The following method offers a means of cleaning common reagent-grade chemicals.

4 Ultrapure water.

5 0.45 μm membrane filters (nylon or polycarbonate).

To purify H_4EDTA

1 Weigh about 100 g H_4EDTA acid and put in a Teflon beaker.

2 Add about 150 mL of 2% HNO_3 trace metal grade.

3 Stir 10 min on magnetic stirrer.

4 Let settle and decant and discard the supernatant.

5 Repeat at least three times with the addition of about 150 mL HNO_3, stir, settle, decant.

6 Rinse with ultrapure water (or equivalent) using the same procedure as above (i.e., add about 150 mL water, stir, settle, decant) at least three times.

7 Dry the prepared chemical in a warm oven (~40°C) overnight (you might have to crush the H_4EDTA before storing).

To prepare pure NH_4OH

Trace metal-grade ammonia can be purchased, but it can also be prepared in the laboratory using reagent-grade ammonia; you need very clean labware and an efficient fumehood.

Under the fumehood, in a very clean desiccator, place a beaker with about 100 mL of concentrated ACS reagent-grade NH_4OH and another Teflon beaker with 100 mL ultrapure water. Replace cover and let stand overnight. The next morning you will have pure 1:1 diluted ammonia in your Teflon beaker.

To prepare purified ammonium EDTA salt

In a 2 L volumetric flask containing about 1.8 L ultrapure water, add 29.2 g purified H_4EDTA. Place on a magnetic stirrer under a fumehood and add about 25 mL of purified 1:1 ammonia prepared as described above. Stir. Continue adding NH_4OH gradually until the H_4EDTA completely dissolves (around pH 6). Adjust to pH 7.0 ($\pm$0.1) and make to volume with ultrapure water. Store in a well stoppered 2 L polyethylene bottle.

10.4.2 Procedure

1 Work at room temperature. Before taking a subsample of soil, make sure your sample is very well homogenized by mixing thoroughly for about a minute. Work in triplicates. Take a subsample of each soil to estimate moisture content. Include two blank solutions (tube and solution without soil) within each batch of extraction. Weigh about 1.000 g of soil in a 50 mL centrifuge tubes and record weight.

2 Add 25 mL of purified 0.05 *M* NH_4-EDTA to each tube, cap and shake on the end-over-end shaker for 1 h at 15 rpm.

3 Centrifuge at 5000 *g* for 10 min, if possible, maintain the temperature of the centrifuge at 20°C, filter through a 0.45 μm membrane, and keep in well-sealed polyethylene bottle at 4°C. Dilute with ultrapure water for analysis. Make sure the standards used for calibration are in the same matrix as the diluted solution.

10.4.3 Calculations

$$\text{M } (\mu\text{g g}^{-1}) = \text{C } (\mu\text{g L}^{-1}) \times 0.025 \text{ L}/(\text{wt. soil g} \times (1 - \text{mc})) \qquad (10.3)$$

where M is the metal content, C is the concentration measured, and mc is the moisture content expressed as a 2-decimal fraction (i.e., 5% = 0.05).

10.4.4 Comments

1 EDTA is a powerful extractant that is capable of extracting significant quantities of trace elements from high affinity sites on the soil surface. Likewise EDTA will extract all elements from the surfaces of plastic and glassware if in contact with the solution. Consequently it is very important to preclean labware with purified 0.5 *M* H_2EDTA followed by a complete water rinse to avoid contamination of the samples.

2 It is also important to use the same matrix for samples and standards. Do not try to acidify the solution before, or while measuring the content of metals as this could cause precipitation of the EDTA.

10.5 HOT ACID-EXTRACTABLE TRACE ELEMENTS (USEPA 1994)

10.5.1 Materials and Reagents

1. Specialized microwave digestion system with Teflon liners
2. Nitric acid (HNO_3)—trace metal grade
3. 100 mL polyethylene volumetric flasks
4. Ultrapure water

10.5.2 Procedure

1. Follow the safety directions from the microwave system manufacturers. Work under a fumehood and wear protective clothing and equipment.
2. Weigh up to 0.500 g of soil sample. If sample contains high content of organics or carbonates, decrease the amount weighed. Organic soils and forest floor horizons should be 0.200 g of sample.
3. Add 10 mL HNO_3. If a strong reaction is observed, allow the samples to stand for several hours (or over night) before sealing the containers to decrease the possibility the containers will vent during heating. Close containers and place in the microwave system. Follow the manufacturer's recommendations for a heating program and maintain a temperature of 185°C for at least 10 min.
4. After completion of the digestion, let cool and transfer the whole sample to a 100 mL volumetric flask (final acidity 10% HNO_3). Let settle overnight and decant supernatant into a 30 mL polyethylene bottle.
5. Dilute five times with ultrapure water for analysis on an ICP-MS (final acidity 2%). Standards should be prepared in the same matrix.

10.5.3 Calculations

$$\text{M } (\mu\text{g g}^{-1}) = \text{C } (\mu\text{g L}^{-1}) \times \text{DF} \times 0.100 \text{ L}/(\text{wt. soil g} \times (1 - \text{mc})) \quad (10.4)$$

where M is the metal content, C is the concentration measured, mc is the moisture content expressed as a 2-decimal fraction (i.e., 5% = 0.05), and DF is the dilution factor.

10.5.4 Comments

Refer to the manufacturer's instructions on the proper use of the microwave digestion system. Due to the high pressures that are developed in the reaction vessels, it is important to use a microwave digestion system designed specifically for this purpose. In addition to the danger of having a vessel explode while being heated, it is also very important to properly cool the vessels before trying to open them. Letting them sit for 30 min in an ice bath is recommended.

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Chapter 11
Readily Soluble Aluminum and Manganese in Acid Soils

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11.1 INTRODUCTION

Approximately 5% of the 3.95 billion ha of acid soils is used for agricultural production while 67% supports forests and woodlands (von Uexküll and Mutert 1995). Plant growth in acid soils is usually limited by low pH and/or Al toxicity. The solubility of Al and Mn in mineral soils increases rapidly when soil pH drops below a value of 5 so that low pH and high soluble Al and Mn concentrations are interrelated. Although the availability of plant nutrients such as P and Ca can be limiting at low soil pH (Foy 1984; Asp and Berggren 1990), Al toxicity is probably the foremost growth-limiting factor in acid soils (Andersson 1988). Root growth, and consequently water and nutrient uptake, are inhibited when dissolved Al attains toxic levels in soil solutions.

Dissolved Al in acid soil solution is typically comprised of monomeric Al ions (e.g., Al^{3+}, $Al(OH)^{2+}$, and $Al(OH)_2^{+}$) as well as organically complexed and polynuclear forms of Al (e.g., $Al_2(OH)_2^{4+}$ and $Al_{13}O_4(OH)_{24}^{7+}$ (Akitt et al. 1972)). Polynuclear and organically complexed Al species are considered to have little, if any, phytotoxicity (Andersson 1988; Wright 1989) although there is some contrary evidence for polymeric Al (Bartlett and Diego 1972; Hunter and Ross 1991). Ideally, the soil solution in the root zone should be analyzed for phytotoxic Al species concentration. For diagnostic purposes, such procedures

would be too time-consuming. Typically, therefore, the soil is extracted with a dilute neutral salt solution that would perturb the ionic equilibrium as little as possible, and for a time period sufficient only to bring into solution readily soluble Al (i.e., not associated with the solid phase). Included in such extracts would be mainly monomeric and polymeric Al and Al complexed by organic ligands of low-molecular weight.

11.1.1 Aluminum and Manganese Toxicity in Agricultural Soils

Aluminum concentration in plant tissues cannot be used to confirm Al toxicity since it does not accumulate in aboveground plant tissues. Although Mn accumulates in plants somewhat in proportion to plant injury in acid soils, its concentration in plant tissues is not a reliable indicator of its toxicity (Foy 1984). Therefore, Al and Mn toxicity diagnostic criteria, especially Al, have been approached through soil analysis. In a meta-analysis of Al toxicity thresholds for crops and forages, Bélanger et al. (1999) found that the total dissolved Al concentrations associated with negative effects in 10% and 50% of the studies were, respectively, 0.003 and 0.02 m*M*. However, different crops and forages, and even varieties within a species, vary in their sensitivity to dissolved Al.

Soil acidity is usually corrected by liming or adding calcium amendments to the soil. The lime requirement (see Chapter 12), i.e., the amount of $CaCO_3$ or its equivalent that has to be applied to the soil to raise its pH to a certain desired value, usually 6.5, can be determined by equilibrating a soil sample with a buffered salt solution and measuring the pH (Shoemaker et al. 1961; McLean et al. 1978). Kamprath (1970) suggested that liming can also be based on soluble Al extracted from acid soils by a neutral unbuffered salt solution, such as 1 *M* KCl, at least for soil groups such as ultisols and oxisols. In Canada, Hoyt and Nyborg (1971a,b, 1972, 1987) showed that crop response on acid soils was closely related to 0.01 *M* and 0.02 *M* $CaCl_2$-soluble Al and Mn. With the exception of alfalfa, yields of the test crops were more closely correlated with dilute $CaCl_2$-extractable Al than soil pH or exchangeable Al (1 *M* KCl-exchangeable) (Webber et al. 1982; Hoyt and Nyborg 1987). There was little response of barley (*Hordeum vulgare* L.), an Al-sensitive crop, to lime when dilute $CaCl_2$-extractable Al approached 1 mg kg^{-1} (Hoyt et al. 1974). Webber et al. (1977) found that the amount of lime required to lower 0.02 *M* $CaCl_2$-extractable Al to 1 mg kg^{-1} was less than the lime requirement to achieve a pH of 6 as determined by the Shoemaker–McLean–Pratt (SMP) procedure (Shoemaker et al. 1961). Research studies in Australia, New Zealand, and the United States also showed that the Al and Mn extracted by dilute $CaCl_2$ solution are suitable diagnostic criteria for Al and Mn toxicities in acid soils (Wright et al. 1988, 1989; Close and Powell 1989; Conyers et al. 1991).

11.1.2 Aluminum and Manganese Toxicity in Forest Soils

Forest decline since the last 20 years in central Europe and eastern North America has been attributed to several environmental stresses such as gaseous pollutant injury and water stress (Hinrichsen 1986). As with crop species, it was also shown that increased Al activity in the soil solution has adverse effects on tree functions and growth (see review by Cronan and Grigal 1995). Forest soils are typically acidic (pH < 5) and thus, the solubility of toxic Al and Mn is generally high. Manganese toxicity to trees was not studied as much. However, as for crop species, foliage Mn status appears to be a better indicator of solution Mn levels compared to Al, but its toxicity is difficult to show due to concomitant high availability of

Al (Hoyle 1972; Kazda and Zvacek 1989). In solution cultures, Hoyle (1972) found that foliage levels of 441 mg Mn kg^{-1} (solution Mn 0.091 m*M*) in yellow birch were optimal for growth but levels above 1328 mg Mn kg^{-1} (solution Mn 0.45 m*M*) were detrimental. In air-polluted European forests, Mn concentrations in soil solutions ranged from 0.018 to 0.36 m*M*, depending on the acid load and parent material type (Kazda and Zvacek 1989).

Joslin and Wolfe (1988) found that dissolved inorganic monomeric Al, total Al, Al^{3+} activity as well as $SrCl_2$-extractable soil Al explained respectively 79%, 74%, 61%, and 61% of the variability in root biomass. The $SrCl_2$-extractable Al and inorganic monomeric Al concentrations at which significant reductions in root branching and fine root biomass was first observed were 10 mg kg^{-1} and 0.1 m*M*, respectively (Joslin and Wolfe 1988, 1989). We used the data from Joslin and Wolfe (1988) to assess how $SrCl_2$-extractable Al and inorganic monomeric Al concentrations are related. Excluding one outlier from the Becket site, 71.2% of the variability in inorganic monomeric Al concentrations (y) can be predicted from $SrCl_2$-extractable Al concentrations (x) using the following power function: $y = 4.70^{0.240x}$. A soil with 10 mg kg^{-1} of $SrCl_2$-extractable Al therefore corresponds to a solution inorganic monomeric Al level of about 0.05 m*M*, which is close to the 0.07 m*M* toxicity threshold obtained from a meta-analysis computed by Bélanger (2000). Finally, Joslin and Wolfe (1989) discussed the unique response of trees at the Becket site (i.e., substantial root growth despite the relatively high foliage Al concentrations and $SrCl_2$-extractable soil Al levels) and suggested that most of the Al absorbed by trees was organically bound. It is known that the complexation of metal cations by organics enhances plant uptake (Arp and Ouimet 1986) but this form is nontoxic to trees (Rost-Siebert 1984). Therefore, the $SrCl_2$ method may not always be a reliable indicator of the potential toxicity of Al in soils where organically bound Al dominates.

11.2 EXTRACTION PROCEDURE FOR AGRICULTURAL SOILS

Hoyt and Nyborg (1972) reported that when Al and Mn in acid soils were extracted with 2.5 to 40 m*M* $CaCl_2$ solutions, generally better correlations with the yield response of three crops were obtained if the extractant was 20 m*M* $CaCl_2$. A subsequent study showed that a 5 min shaking was adequate and gave only slightly lower concentrations of Al than a 1 h shaking and twice the Al concentration as 10 m*M* $CaCl_2$ (Hoyt and Webber 1974). According to Webber et al. (1977), liming is likely not needed for Canadian acid soils when extracted Al is 1 mg kg^{-1} or less. Close and Powell (1989) also used this extraction procedure for New Zealand soils.

11.2.1 Materials and Reagents

1. 0.02 *M* $CaCl_2$: Dissolve 5.88 g of reagent-grade $CaCl_2 \cdot 2H_2O$ in about 250 mL of deionized water and dilute to 2 L.
2. 50 mL centrifuge tubes and rubber stoppers.
3. Whatman No. 42 filter paper or equivalent.
4. Centrifuge, with rotors accepting 50 mL centrifuge tubes.
5. Reciprocal shaker.
6. Liquid dispenser.

11.2.2 Procedure

1. Weigh 10 g of soil (<2mm) into centrifuge tube.
2. Dispense 20 mL of the 0.02 *M* $CaCl_2$ reagent into the centrifuge tube and stopper tightly.
3. Shake 5 min on the shaker (120 oscillations min^{-1}).
4. Remove from shaker and centrifuge at 1250 *g* for 1 min to facilitate rapid filtering.
5. Filter through a fluted filter paper into receptacle for storing the extract.

11.2.3 Comments

A 0.01 *M* $CaCl_2$ solution is closer in ionic strength to the soil solution of agricultural soils than is a 0.02 *M* solution and it is also commonly used to assess other soil chemical properties such as pH and soluble P (Soon 1990). Therefore, it may be advantageous to use 0.01 *M* instead of 0.02 *M* $CaCl_2$ solution when those soil properties are also to be determined. However, a critical level of 0.01 *M* $CaCl_2$-soluble Al has not been proposed. The above procedure has not been tested for soils with organic matter content much higher than 10%.

11.3 EXTRACTION PROCEDURE FOR FOREST SOILS

Since increased Ca availability alleviates toxic effects of Al on trees (i.e., Al toxicity is mostly indirect—it is toxic due to its antagonistic effects on divalent cation uptake (Cronan and Grigal 1995)), the $SrCl_2$ method is advantageous relative to the commonly used $CaCl_2$ procedure in agricultural soils as it allows the quantification of extractable Ca and other cations as well as Ca and Mg to Al ratios. Strontium is slightly more efficient at displacing Al than Ca, but the difference is only about 5% at this ionic strength (0.01 *M*). However, no work has been done on the relative amounts of Al and Mn exchanged with $SrCl_2$, $CaCl_2$, and $BaCl_2$. Such a study would help to clarify the need for using different extractants when the amount of potentially toxic elements was under investigation. The $SrCl_2$ method of Joslin and Wolfe (1989) is described here after some modifications based on the suggestions of Heisey (1995).

11.3.1 Materials and Reagents

1. 0.01 *M* $SrCl_2$: Dissolve 5.332 g of reagent-grade $SrCl_2 \cdot 6H_2O$ in about 250 mL of distilled/deionized water and make to volume in a 2 L volumetric flask.
2. 50 mL centrifuge tubes and screw caps.
3. Ultracentrifuge accepting 50 mL tubes.
4. End-over-end shaker.
5. Pipette and liquid dispenser.

11.3.2 Procedure

1 Weigh 10 g of soil (dried and <2mm) into centrifuge tube.

2 Add 20 mL of 0.01 *M* $SrCl_2$ to the centrifuge tube.

3 Shake for 60 min at 15 oscillations min^{-1}.

4 Remove from shaker and centrifuge for 30 min at 7000 *g*.

5 Pipette off the supernatant and retain in container for analysis.

11.3.3 Comments

An end-over-end shaker is used here because it is more efficient in wetting and mixing forest soils with a high litter/organic matter content and using a low soil:extractant ratio. The low soil:extractant ratio results in a thick suspension that typically requires a high-speed centrifuge to separate.

11.4 DETERMINATION OF ALUMINUM

Aluminum in the extracts can be measured by atomic absorption (Webber 1974), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Carr et al. 1991), or by colorimetry (Hoyt and Webber 1974; Carr et al. 1991). Atomic absorption or ICP-AES will give results for total dissolved Al whereas colorimetry should yield results for Al ''reactive'' with the chromogen. What will be included as ''reactive'' Al depends on the equilibration time allowed for color development. Shorter reaction times should yield mainly labile (monomeric) Al whereas longer reaction times would include the determination of polymeric and complexed Al. Grigg and Morrison (1982) showed that the pyrocatechol violet (PCV) method was superior to the Aluminon (aurintricarboxylic acid triammonium salt) method in precision, and automating the procedure resulted in further improvement in its precision. The pyrocatechol method was also recommended by Conyers et al. (1991). The method below is an adaptation of Wilson and Sergeant (1963). The procedure is simple and reliable.

11.4.1 Reagents

1 0.1% (w/v) PCV. Keep in a dark glass bottle.

2 0.1% (w/v) *o*-phenanthroline (OP). Store in a polyethylene bottle.

3 10% (w/v) hydroxylamine hydrochloride (HH). Keep in a polyethylene bottle.

4 10% (w/v) ammonium acetate (buffered at pH 6.2 using acetic acid). Store in a polyethylene bottle.

5 Aluminum working standard solution: from a stock standard solution containing 1 g Al L^{-1}, prepare a working standard containing 100 mg Al L^{-1} in $CaCl_2$

solution of the same molarity as the soil extractant. By further dilution, prepare 5 standards over the range of 0.1 to 2.5 mg Al L^{-1} in $CaCl_2$ solution of the same molarity. For forest soils, standards should be prepared in 0.01 *M* $SrCl_2$.

11.4.2 Procedure

1 Pipette 2 mL of extract or standard solution into 16 mm × 125 mm culture tubes. The tubes should be prewashed with 0.1 *M* HCl. Sample solutions should contain no more than 5 μg Al.

2 Add sequentially 0.5 mL each of PCV, OP, and HH, gently swirling the contents of the tube after each addition. In a batch of samples, each reagent should be added to all samples before adding the next reagent.

3 Add 6 mL of the buffer solution, stopper and invert the tube three times and allow to stand for 1 h.

4 Measure absorbance at 580 nm with a spectrophotometer using 1 cm cuvette. Plot the absorbance values against μg Al. The μg Al value read off the calibration curve gives extracted Al level in mg kg^{-1} soil. If dilution of the extract is required, multiply by the dilution factor.

11.4.3 Comments

The extracts should be analyzed with minimum delay. If delays are inevitable, acidify the samples slightly to prevent polymerization of Al monomers. The PCV powder and the prepared solution should be kept in the dark in tightly sealed containers. Interference by iron is diminished by the OP and HH reagents. Color development is maximal and stable between 1 and 2 h, after which the color gradually declines. Reagent blank values are determined using the soil extractant. It is advisable to use freshly prepared PCV solution. The other reagents are stable for at least 4 weeks when stored at room temperature.

The analysis as described should include monomeric and polymeric Al and weakly complexed Al. Kerven et al. (1989) described a PCV procedure with a reaction time of 60 s to measure only monomeric Al. For forest surface soils, which typically have much higher organic matter content than agricultural soils, the difference between measuring total dissolved Al and monomeric inorganic Al should be more critical. Also much calibration of extractable soil Al with crop response has been done using dissolved total Al (Hoyt and Webber 1974; Hoyt and Nyborg 1987). An autoanalyzer PCV method that uses ion-exchange to separate inorganic monomeric Al from organically complexed Al has been described by McAvoy et al. (1992).

11.5 DETERMINATION OF MANGANESE

Manganese in the soil extract is determined by atomic absorption spectrometry using an oxidizing air–acetylene flame. ICP-AES analysis would also be convenient, especially if Al is to be determined.

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Chapter 12
Lime Requirement

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12.1 INTRODUCTION

The soil pH indicates the amount of acidity present in the soil solution and is one of the most commonly measured soil properties. It is considered as a standard and routine soil analysis. Soil pH affects the solubility and availability of many elements as well as microbial activity (Curtin et al. 1984; Marschner 1995). An acid soil commonly has concentrations of Al or Mn that are high enough to be toxic to some plants. The target soil pH, which represents the soil pH value associated with optimum plant growth, varies with crop species and can be influenced by soil type. In general, a soil pH of 6.0 to 7.0 is ideal for most agronomic crops such as corn (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and wheat (*Triticum aestivum*). However, a lower target pH may be acceptable for other plants such as potato (*Solanum tuberosum* L.) or blueberry (*Vaccinium* spp.). Liming acid soils to maintain an appropriate pH for plants is, therefore, an essential practice for soil and crop management in many areas.

There are two components of soil acidity that are used in determining lime application: active acidity and exchangeable (reserve) acidity. Active acidity is the concentration of H^+ ions in the soil solution phase and indicates whether or not liming is required to reduce soil acidity. The exchangeable acidity refers to the amounts of H^+ ions present on exchange sites of clay and organic matter fractions of the soils and affects the amount of lime needed to achieve the target soil pH. The greater the exchangeable (reserve) acidity, the more the soil is said to be buffered against change in pH and the greater the lime requirement (LR).

Lime requirement is defined as the amount of agricultural limestone ($CaCO_3$), or any other basic material, required to increase soil pH from acidic conditions to a target level that is optimum for the desired use of the soil. The nature of soil acidity, along with soil physical and chemical properties (mainly soil texture and organic matter content), affects the LR. The test used such as soil–lime incubations, soil–base titrations, or soil–buffer equilibrations can also affect the recommendation for lime (Aitken 1990; Conyers et al. 2000; Alatas et al.

2005). Accurate methods to assess the amount of liming materials are essential, and different LR tests should be used in different geographical areas based both on research and practical experience. The selection of one specific technique to determine LR must also be taken into consideration some practical aspects such as the time available to conduct the test, the required equipment and supplies, the cost, etc. Many techniques and methods have been developed and successfully used worldwide to measure LR and are reported in previous studies (McLean 1982; van Lierop 1990). The majority of these methods are based on the following principles (Sims 1996): (i) the measured LR should reflect the amount of liming material needed to reach the target pH when the lime is applied under field conditions; (ii) LR test should accurately measure all forms of acidity (dissociated and undissociated) present in a soil; (iii) LR test should be calibrated in the geographic area where the test will be used; and (iv) LR test should be calibrated to determine conversion factors between limestone and the other liming materials used.

To estimate the amount of lime required to correct soil acidity and attain a desired soil pH, different procedures can be used through field or laboratory studies. Soil–lime incubations, soil–base titrations, and soil–buffer equilibrations (Viscarra Rossel and McBratney 2003; Machacha 2004; Liu et al. 2005) are the most commonly used methods. Estimation of LR based on field studies, however, remains the most accurate means to determine LR for a soil, and especially to evaluate new liming materials. Although these methods are time consuming and expensive, they are the foundation for the more rapid and inexpensive procedures. In routine soil testing laboratories in North America, the Adam–Evans (A–E) buffer (Adams and Evans 1962) and the Shoemaker–McLean–Pratt (SMP) (Shoemaker et al. 1961) procedures are the most commonly used methods. In Canada for example, Nova Scotia and Newfoundland currently use the A–E procedure while New Brunswick, Prince Edward Island, Ontario, British Columbia, and Quebec use the SMP method. Webber et al. (1977) recommend the SMP method for Canadian acid soils. Tran and van Lierop (1982) and van Lierop (1983) also found the method to be suitable for acid mineral and organic soils in Quebec. Recently, Warman et al. (2000) recommended the replacement of the A–E method with the SMP method in Nova Scotia and Newfoundland. For these reasons, only the SMP method is described in this chapter.

12.2 SHOEMAKER–McLEAN–PRATT SINGLE-BUFFER METHOD

12.2.1 Principles

The SMP method was developed in 1961 from a soil–lime ($CaCO_3$) incubation study using 14 acidic soils from Ohio (Shoemaker et al. 1961). The accuracy of this procedure relies on its calibration of decreasing soil–buffer pH values with increasing LR rates. Originally, this procedure was particularly well adapted for determining the LR of soils needing LR >4.5 Mg ha^{-1}, and with pH values <5.8 and organic matter contents <100 g kg^{-1} (McLean 1982). van Lierop (1990) improved the accuracy of the SMP single-buffer method at low LR values and proposed the amount of lime required to attain target values of 5.5, 6.0, 6.5, and 7.0 (Table 12.1). This improvement is obtained by fitting curvilinear instead of linear equations to the relationships between soil–buffer pH and incubation LR values and is based on a number of LR studies (McLean 1982; Soon and Bates 1986; Tran and van Lierop 1993).

TABLE 12.1 Relationships between Soil SMP-Buffer pH and Lime Requirement Values to Achieve pH 5.5, 6.0, 6.5, and 7.0 of Mineral Soils

	Quantity of liming material (Mg ha^{-1}) required to reach desired pH			
Soil–buffer pH	**5.5**	**6.0**	**6.5**	**7.0**
6.9	0.5	0.6	0.7	0.9
6.8	0.6	1.0	1.2	1.5
6.7	0.7	1.4	1.8	2.2
6.6	0.9	1.8	2.5	2.8
6.5	1.2	2.3	3.3	3.6
6.4	1.6	2.9	4.0	4.4
6.3	2.0	3.5	4.9	5.2
6.2	2.5	4.2	5.7	6.0
6.1	3.1	4.9	6.6	7.0
6.0	3.8	5.6	7.5	8.0
5.9	4.5	6.5	8.5	9.0
5.8	5.3	7.3	9.5	10.0
5.7	6.1	8.2	10.5	11.2
5.6	7.0	9.2	11.6	12.4
5.5	8.0	10.2	12.7	13.6
5.4	9.1	11.3	14.0	14.9
5.3	10.2	12.4	15.0	16.2
5.2	11.4	13.6	16.2	17.6
5.1	12.7	14.8	17.5	19.0
5.0	14.0	16.1	18.8	20.4
4.9	15.5	17.4	20.1	22.0

Source: From van Lierop, W., in R.L. Westerman (Ed.), *Soil Testing and Plant Analysis*, 2nd ed., SSSA, Madison, Wisconsin, 1990, 73–126.

Lime requirement in Mg $CaCO_3$ for a furrow layer of 20 cm depth of soil.

12.2.2 Materials and Reagents

1. pH meter
2. Disposable plastic beakers
3. Automatic pipette
4. Glass stirring rods
5. Mechanical shaker
6. Standard buffers, pH 7.0 and 4.0
7. SMP buffer solution
8. 0.1 *M* HCl, 4.0 *M* NaOH, and 4.0 *M* HCl solutions

The SMP buffer solution can be prepared as follows:

a. Weigh and place in a 10 L bottle the following chemicals:
   - 18 g *p*-nitrophenol ($NO_2C_6H_4OH$);
   - 30 g potassium chromate (K_2CrO_4);
   - 531 g calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$).

b. Add approximately 5 L of distilled water. Shake vigorously as the water is added, and continue shaking for a few minutes to prevent formation of a crust over the salts.

c. Dissolve 20 g of calcium acetate [$(CH_3COO)_2Ca \cdot H_2O$] in a separate flask containing about 1 L of distilled water.

d. Add solution from step (c) to that from step (b) and continue shaking for about 2 or 3 h.

e. Add 100 mL of dilute triethanolamine (TEA) solution: TEA ($N(CH_2OH)_3$) is very viscous and difficult to pipette accurately. It is recommended that a dilute TEA solution be prepared by diluting 250 mL (or 280.15 g) of TEA to 1 L with distilled water and mix well.

f. Shake the mixture periodically until it is completely dissolved. This takes about 6 to 8 h.

g. Dilute to a final volume of 10 L with distilled water.

h. Adjust pH to 7.5 ± 0.02 by titrating with either 4 *M* NaOH or 4 *M* HCl as required.

i. Filter through fiberglass sheet or cotton mat if necessary.

j. Verify buffer capacity of prepared SMP buffer by titrating 20 mL from pH 7.5 to 5.5 with 0.1 *M* HCl. This should take 0.28 ± 0.005 cmol (+) HCl/pH unit.

The 10 L SMP prepared solution can be used for approximately 500 soil samples.

12.2.3 Procedure

1 Measure 10 mL or weigh 10 g air-dried, screened (<2 mm) soil samples in appropriate beakers.

2 Add 10 mL of distilled water and stir with glass rod and repeat stirring periodically during the next 30 min.

3 Measure the soil pH in the beaker (soil + H_2O) and rinse electrodes with a minimum of distilled water.

4 If the soil pH (H_2O) is less than the desired pH, add 20 mL of SMP buffer to the soil–water mixture (soil–water–buffer ratio is 1:1:2 by volume) and stir with glass rod.

5 Place soil–water–buffer samples on a mechanical shaker for 15 min at about 200 oscillations min^{-1}. Remove samples from shaker and let stand for 15 min. The times of shaking and standing are very important and should be respected. Sims (1996) proposed 30 min of shaking and 30 min of standing.

6 Adjust the pH meter to read 7.5 with SMP buffer.

7 Stir sample thoroughly and read the soil–water–buffer to nearest 0.01 pH unit. Record as soil–buffer pH.

8 Select the amount of lime required to bring the soil to the pH you choose to lime the soil, based on soil–buffer pH relationships used in local recommendations (e.g., CRAAQ 2003; OMAFRA 2003).

9 As the SMP buffer solution can affect the accuracy of the glass electrode after approximately 200 buffer–pH determinations, it is strongly recommended to regenerate the electrodes by appropriate procedure. The combined glass electrode can be regenerated by immersing it into a plastic beaker containing a solution of 10% ammonium hydrogen fluoride ($NH_4F \cdot HF$) for 1 min. Since the $NH_4F \cdot HF$ is a hazardous compound, appropriate protection should be respected according to its Material Safety Data Sheet. After etching, dip electrode into 1:1 H_2O–HCl solution to remove silicate. Rinse the electrode thoroughly with distilled water and immerse in hot 3 *M* KCl solution (50°C) for 5 h. The electrolytes in the electrode (saturated KCl or calomel) must be replaced if necessary.

12.3 COMMENTS

For a LR greater than about 7 Mg limestone ha^{-1}, it is recommended to divide the rate into two or more applications to avoid local overliming (Brunelle and Vanasse 2004). This is important as a liming recommendation assumes that the material is homogeneously incorporated into the plow-layer, a precept that is difficult to achieve in practice. When surface applying liming material, without significant incorporation (i.e., without tillage), the rate should be reduced to about a third. Where some tillage is practiced, but not to the typical plow-layer depth used in the calibration of the test, then the liming rate should be reduced proportionately (van Lierop 1989).

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Chapter 13
Ion Supply Rates Using Ion-Exchange Resins

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13.1 INTRODUCTION

Use of ion-exchange resins to measure nutrient availability in soils was reported as early as 1951 (Pratt 1951) and 1955 (Amer et al. 1955). Since then, many journal articles have been published on the use of ion-exchange resins in agricultural and environmental soil research, mostly focusing on measuring nutrient availability in soil. Anion-exchange resin extraction as a method to assess P availability in soil has been described earlier by Olsen and Sommers (1982) and Kuo (1996). The principle of resin membrane extraction is also briefly described and commented on by Havlin et al. (2005). A review of application of ion-exchange resins in agricultural and environmental research has been provided by Qian and Schoenau (2002a).

Synthetic ion-exchange resins are solid organic polymers with an electrostatic charge that is neutralized by a selected counterion of opposite charge, and hence they function in a manner analogous to charged soil colloids. The strongly acidic cation-exchange (sulfonic acid functional group) resins and strongly basic anion-exchange (tertiary ammonium functional group) resins are chosen for use as a sink to extract nutrient ions in soils and other media. When ion-exchange resins are equilibrated with a solution containing a mixture of ions, proportions adsorbed by resin will not be the same as ionic proportions in the bulk solution, because of preferential selectivity by the resins for various ions. Generally speaking, cations and anions with the lowest affinity to the resin are best for use as counterions. There are two forms of ion-exchange resins that are commercially available. One is bead form and the other is membrane form. The resin beads are normally retained in a sealed nylon bag, while the

resins in membrane form should be cut into the desired size of strips (Qian et al. 1992) before use. Both resin beads and membranes have evolved from initial usage in batch systems where beads or membranes are mixed with a certain amount of soil and water, and then shaken as a suspension for a fixed time period (Amer et al. 1955; Martin and Sparks 1983; Turrion et al. 1999) to diffusion-sensitive systems where ion-exchange resins are placed in direct contact with soil for extended periods (Skogley 1992; Ziadi et al. 1999; Qian and Schoenau 2002b). When ion-exchange resins are used in the diffusion-sensitive systems, it is not easy to place them in the soil *in situ*, especially for resin membrane strips. To overcome the difficulty, resin capsule (made by sealing the resin bead inside a porous shell to form a compact rigid sphere capsule) and PRS probe (made by encapsulating the membrane in a plastic frame to create a probe) are commercially available from UNIBEST (Bozeman, Montana) and Western Ag Innovations (Saskatoon, SK), respectively.

In batch systems, the resins are in aqueous suspension with soil. During extraction, the resins adsorb nutrient ions from soil solution via surface adsorption, and the resins maintain ion concentrations at a low level to facilitate continued nutrient ion desorption from the soil until equilibrium is reached (Sparks 1987). In diffusion-sensitive procedures, resins are placed in direct contact with soil, which provides a measure that includes both the rates of release of ions from different soil surfaces as well as their diffusion rates through bulk soil. The system integrates both chemical and biological transformations as well as diffusion to a sink into the measure of nutrient availability, which accounts for the kinetics of nutrient release and transport (Curtin et al. 1987; Abrams and Jarrel 1992). With its nature of action similar to a plant root in its extraction of nutrient ions in soils, this method is able to account for factors affecting nutrient uptake by plant roots (Qian and Schoenau 1996). The theoretical verification for the procedures has been documented previously (Yang et al. 1991a,b; Yang and Skogley 1992).

With diffusion-sensitive systems, we can easily measure the nutrient supply rate (NSR). The NSR is defined as the amount of nutrient ion adsorbed per unit surface area of resin membrane over the time of duration of direct contact with soil. It can be expressed as μg (or μmol) per cm^2 for the time of direct contact (i.e., 24 h). There is no direct calibration between supply rate data and soil nutrient concentrations determined by conventional extractions as they are different measurements. Using ion-exchange resin membrane in contact with soil to assess nutrient availability is an alternative approach to traditional chemical extractions in that it provides a measure of nutrient ion flux, and is useful in mimicking and tracking the dynamic behavior of ion supply to plant roots in soil (Qian and Schoenau 2002a). It can be considered a unique multiple element assessment that is universal in its application to soils from different regions and of different properties.

Current efforts in assessing nutrient ion supply rate in soil have focused on direct contact of resin with soil either in the laboratory or in the field (Qian and Schoenau 2002a). The embodiment of resin membranes into probes facilitates the use of ion-exchange resins *in situ* in the field or fresh bulk soil samples in the laboratory. A so-called ''sandwich test'' for laboratory testing can be used to measure NSR in soil, which only requires a few grams of soils, and is suitable for soil samples that have been ground and dried in preparation for other types of analysis. The ''sandwich'' test for laboratory testing is described in this chapter.

13.2 LABORATORY MEASUREMENT OF NUTRIENT (ION) SUPPLY RATE—''SANDWICH'' TEST

13.2.1 Principle

The ''sandwich'' test was developed to use a minimum amount of processed (air-dried and ground) soil to achieve a measurement of NSR. The basic principle is to allow the resin membrane to adsorb nutrient ions from soils by directly contacting it with the soil in a moist condition for 24 h.

13.2.2 Materials and Reagents

1. Resin membrane: supplied from Western Ag Innovation Inc. (Saskatoon, SK). Other sources are BDH (Poole, England) and Ionics (Watertown, Massachusetts). The membrane sheets are cut into squares of about 8 cm^2 each to ensure the square is of a size that just fits inside the vial cap.
2. Snapcap vial lids (7 dram).
3. Snapcap vials with lids (7 dram).
4. Parafilm laboratory film.
5. Analytical balance.
6. Shaker.
7. Pipette (1 or 2 mL) and tips (or dropper).
8. 0.5 *M* $NaHCO_3$ solution: dissolve approximately 42 g of $NaHCO_3$ in deionized water and make to volume in a 1 L volumetric flask.
9. 0.5 *M* HCl: mix 42 mL of concentrated HCl with deionized water and make to volume in a 1 L volumetric flask.

13.2.3 Procedure

Preparation/Regeneration

The resin membranes must be cleaned and regenerated before each use. It is very important that used membrane strips are not contaminated with ions of interest before making measurements.

1. Before use, cation-exchange resin membranes must be cleaned/regenerated by soaking in 0.5 *M* HCl twice, for 1 h each time, with 3 mL of HCl per 1 cm^2 of membrane strip. This will put the cation-exchange membrane exchange sites into the proton (H^+) form as the counterion for exchange. The mixture should be stirred or agitated every 15 min or if possible, shaken continuously at slow

speed on a rotary-bench or side-to-side shaker. When the counterions are not protons, the cleaning process should be repeated as many as four times.

2 Clean brand new or regenerate used anion-exchange membranes by soaking in 0.5 *M* $NaHCO_3$ solution four times, for 2 h each time, with 3 mL of $NaHCO_3$ solution per 1 cm^2 of membrane strip. The solution should also be stirred on a regular basis or slowly shaken. This will put the anion-exchange membrane exchange sites into the bicarbonate (HCO_3^-) form.

3 Rinse cleaned or regenerated membrane strips with deionized water, and keep them in deionized water before use.

Extraction

1 Place subsamples of air-dried soil <2 mm into two Snapcap vial lids, filling the lids with soil up to the edges to ensure good contact between the complete surface of the membrane and the soil.

2 Place the Snapcap vial lids with the soil sample on an analytical balance. Add deionized water until the soil in each lid is close to saturated or at field capacity. If adding water just to field capacity, the field capacity of the soil should be estimated in advance to determine how much water is required for the weight of soil used.

3 Place a cation- or anion-exchange membrane strip onto the surface of the soil in one Snapcap vial lid, and then cover with the other Snapcap vial lid, making a ''sandwich,'' with the membrane sandwiched between the two lids containing soil. Normally a cation-exchange membrane sandwich and an anion-exchange membrane sandwich would be made if measurement of all cations and anions is desired.

4 Seal the ''sandwich'' with Parafilm laboratory film to avoid loss of soil moisture during the extraction.

5 Extraction time is normally set at a period of 24 h, similar to the burial time of membranes used in a commercial laboratory.

Elution

1 Add 20 mL of 0.5 *M* HCl to the Snapcap vial (7 dram).

2 Remove the Parafilm from the ''sandwich,'' separate the lids and pick out the membrane strip with plastic tweezers and then wash with deionized water until all soil particles are removed from the membrane surface. It is important that all soil particles are removed to avoid any soil entering into the eluent (HCl).

3 Place the washed membrane strips into the vials with 0.5 *M* HCl. Cap the vials with lids and then shake the vials containing the membranes in a shaker at 200 rpm for 1 h. The cation-exchange membrane and anion-exchange membrane strip from the same sample of soil can both be placed into the same 20 mL of HCl eluent as protons will elute the cations and Cl^- will elute the anions. The eluent should completely cover the membrane strips during shaking to ensure complete elution.

Ion Measurement

Nutrient ion concentrations in HCl can be measured with various instruments commonly used in a soil analytical chemistry laboratory, including manual or automated colorimetry, ion chromatography, atomic absorption-flame emission (AA-FE) spectrometry, or inductively coupled plasma (ICP) spectrometry.

13.2.4 Calculation

NSR can be calculated as

$$\text{NSR} = (C \times V)/S \tag{13.1}$$

where C is the concentration of an adsorbed cation or anion (μg mL^{-1}) in HCl eluent, V is the volume of eluent (mL), and S is the surface area of membrane strip (cm^2).

Example: a ''sandwich'' was prepared with an 8 cm^2 anion-exchange membrane. After 24 h the resin membrane was removed, washed, and placed in 20 mL of 0.5 *M* HCl. The concentration in 0.5 *M* HCl was 10 μg NO_3-N mL^{-1} as measured by colorimetry. The value of NSR is reported as: (10 μg mL^{-1} $\times$ 20 mL)/8 cm^{-2} = 25 μg cm^{-2}.

13.2.5 Comments

1. Ion-exchange resin membranes are a very sensitive measure of nutrient supply. Thus, maintaining consistent and uniform contact between soil and membrane is an essential condition to achieve reproducible results. If there is incomplete contact between the membrane and the soil, the area of membrane surface that can actually adsorb ions from soil is different than that assumed in the calculation of supply rate.

2. The tests should be under the same moisture and temperature conditions. Moisture and temperature have significant effects on ion diffusion and mineralization/solubilization in soil.

3. The ''sandwich'' test requires only a small amount of air-dried soil (about 4.5 g per 7 dram vial lid or 9 g per ''sandwich''). As such, there is no need to prepare a large amount of sample.

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Chapter 14 Environmental Soil Phosphorus Indices

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14.1 INTRODUCTION

The loss of phosphorus (P) in agricultural runoff and its input to freshwater bodies is known to accelerate eutrophication (Carpenter et al. 1998; U.S. Geological Survey 1999; Sharpley 2000). As eutrophication of surface water impairs its use for recreation, drinking, and commercial fishing, several strategies have been put in place to minimize impairment by reducing the potential for P loss from agricultural operations (Gibson et al. 2000; U.S. Environmental Protection Agency 2004).

Key components of remedial strategies to decrease P loss from agriculture are the determination of soil P levels that are above those required for optimum crop growth, due to the continual application of P (Sims et al. 1998; Simard et al. 2000; Daverede et al. 2003) and the identification of critical source areas where there is a high risk of P loss due to the coincidence of runoff and erosion with high soil P levels (Sharpley et al. 2001, 2003; Coale et al. 2002). Traditional soil P tests to estimate for crop P availability have been used as surrogate estimates of runoff P enrichment by soil P (Sharpley et al. 1996). Because soil P tests were developed to work on certain soil types (e.g., Mehlich-3 and Bray-1 for acidic soils and Olsen for calcareous, alkaline soils) and do not mimic soil P release to runoff water, efforts have been made to establish environmental soil P tests (Sibbesen and Sharpley 1997; Torbert et al. 2002). The more prominent of these environmental tests include water-extractable soil P and P sorption saturation.

Considerable field-based research has provided data to support the use of water-extractable soil P as an environmental test, which is independent of soil type, to assess the potential for soil to enrich runoff with dissolved P (Pote et al. 1996; McDowell and Sharpley 2001). The extraction of soil with water more closely mimics the interaction between surface soil and rainfall and the subsequent release of P to runoff water than do acidic or basic soil test P extractants. Andraski and Bundy (2003), Andraski et al. (2003), Daverede et al. (2003), Hooda et al. (2000), Pote et al. (1999a,b), and Torbert et al. (2002) all reported water-extractable soil P to be closely related to runoff-dissolved P for both grassed and cropped plots, at a similar or greater level of significance than Bray-1 and Mehlich-3-extractable soil P (Vadas et al. 2005). Increasingly, investigators are using water-extractable P in lieu of runoff data in laboratory studies aimed at comparing environmental and agronomic effects (e.g., Stout et al. 1998).

Estimation of P sorption saturation is based on the premise that the saturation of P sorbing sites for a soil determines P release (intensity factor) as well as the level of soil P (capacity factor) (Breeuwsma and Silva 1992; Kleinman and Sharpley 2002). For example, soils of similar soil test P may have differing capacities to release P to runoff, based on the fact that P would be bound more tightly to clay than sandy soils (Sharpley and Tunney 2000). Phosphorus sorption saturation can also represent the capacity of a soil to sequester further P addition and thereby enrich runoff P (Schoumans et al. 1987; Lookman et al. 1996). For example, the addition of P to a soil with a high P sorption saturation will enrich runoff P more than if P was added to a soil with a low P sorption saturation, independent of soil test P level (Sharpley 1995; Leinweber et al. 1997). Traditional techniques to estimate soil P sorption saturation have relied upon methods that are not commonly performed by soil testing laboratories, such as acid ammonium oxalate extraction in the dark (e.g., Schoumans and Breeuwsma 1997) and P sorption isotherms (e.g., Sharpley 1995). Recent research has shown soil P sorption saturation in acidic soils can be reliably estimated from Mehlich-3-extractable Al and Fe (primary components of P sorption) and P (Beauchemin and Simard 1999; Kleinman and Sharpley 2002; Nair and Graetz 2002).

Soil P sorption has also been used to estimate the potential of a soil to sequester proposed additions of P. In specific cases, a detailed assessment of the P sorption capacity of a soil is a planning requirement of proposed land applications of biosolids, in order to determine the potential for P leaching through a soil profile (U.S. Environmental Protection Agency 1993; Bastian 1995). Traditionally, P sorption isotherms are constructed using batch equilibrations of soil with P added in a supporting solution, usually as KH_2PO_4 in a 0.01 M $CaCl_2$ matrix for 24 to 40 h (Syers et al. 1973; Nair et al. 1984). Equations such as the Langmuir, Freundlich, and Tempkin models have been used to describe the relationship between the amount of P sorbed to the P in solution at equilibrium and to calculate P sorption maximum, binding energy, and equilibrium P concentrations for a given soil (Berkheiser et al. 1980; Nair et al. 1984). This chapter will discuss the Langmuir approach only.

While P sorption isotherms can provide a large amount of soil-specific information that is useful to agronomic and environmental characterization of P sorption capacity, they are too time-consuming, complicated, and expensive for routine use by soil testing laboratories (Sharpley et al. 1994). To overcome these limitations, Bache and Williams (1971) suggested a single equilibration using a high concentration of P (single-point isotherm), from which a P sorption index (PSI) was calculated, to rapidly determine soil P sorption capacity. They found that PSI was closely correlated with P sorption maxima determined by the full sorption isotherm for 42 acid and calcareous soils from Scotland ($r = 0.97$; $P > 0.001$) (Bache and Williams 1971). Other researchers have subsequently found PSI to be correlated with

soil P sorption maxima of soils varying widely in chemical and physical properties (Sharpley et al. 1984; Mozaffari and Sims 1994; Simard et al. 1994).

Finally, most states in the United States have now adopted a P indexing approach as part of P-based nutrient management planning requirements, so that areas at greatest risk of P loss can be targeted for remediation or more restrictive management (Sharpley et al. 2003). The P indexing approach is based on the knowledge that most P loss from agricultural watersheds (>75% annually), occurs from small, defined areas of a watershed (<20% land area) (Smith et al. 1991; Schoumans and Breeuwsma 1997; Pionke et al. 2000). The P index ranks these critical source areas by identifying where high P source potential (i.e., soil P and the rate, method, timing, and type of P added as fertilizer or manure) coincides with high transport potential (i.e., surface runoff, leaching, erosion, and proximity to a stream) (Lemunyon and Gilbert 1993). The P index is one of the more successful approaches that addresses P source, management, and transport in a holistic way by attempting to combine important P loss variables into a practical program that assesses specific field's potential for P loss (Gburek et al. 2000; Sharpley et al. 2003). Use of the P index helps farmers, consultants, extension agents, and livestock producers identify (i) agricultural areas or practices that have the greatest potential to accelerate eutrophication and (ii) management options available to land users that will allow them flexibility in developing remedial strategies.

This chapter details the methods used to estimate water-extractable soil P, P sorption saturation, P sorption capacity, and indexing P loss potential for a given site. For all these chemical methods and preparation of reagents used, the use of standard laboratory protective clothing and eye covering is recommended.

14.2 WATER-EXTRACTABLE SOIL P

The extraction of soil P with water provides a rapid and simple means of determining the amount of soil P that can be released from soil to runoff water. The method assumes that extraction with water replicates the reaction between soil and runoff water and is thus, independent of soil type. The following method is a variation of the method described by Olsen and Sommers (1982) for determination of water-soluble P in soils. In summary, P extracted from a soil sample after it has been shaken with water for a specific period of time is measured spectrophotometrically by the colorimetric molybdate–ascorbic acid method (Murphy and Riley 1962). Alternatively, filtrates can be analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES), which will measure total dissolved P.

14.2.1 Materials and Reagents

1 Centrifuge tubes (40 mL) with screw caps.

2 End-over-end shaker.

3 Centrifuge.

4 Filtration apparatus (0.45 μm pore diameter membrane filter or Whatman No. 42).

5 Photometer: Spectrophotometer with infrared phototube for use at 880 nm and providing a light path of at least 2.5 cm, preferably a 5 cm path length cell.

For light path lengths of 0.5, 1.0, and 5.0 cm, the P ranges are 0.3–2.0, 0.15–1.30, and 0.01–0.25 mg L^{-1}, respectively.

6 Acid-washed glassware and plastic bottles: Graduated cylinders (5 to 100 mL), volumetric flasks (100, 500, and 1000 mL), storage bottles, pipets, dropper bottles, and test tubes or flasks for reading sample absorbance. The spectrophotometer should be calibrated daily by using factory standard procedures for the laboratory machine.

7 Balances used to weigh reagents and samples are calibrated according to factory specifications and routinely cleaned to ensure proper and accurate working order.

8 Distilled water.

9 A series of P standards (0, 0.25, 0.5, 0.75, and 1.00 mg P L^{-1} as KH_2PO_4) is prepared fresh on the day of analysis.

10 Reagents for ascorbic acid technique for P determination.

a. 2.5 *M* H_2SO_4: Slowly add 70 mL of concentrated H_2SO_4 to approximately 400 mL of distilled water in a 500 mL volumetric flask. After the solution has cooled, dilute to 500 mL with distilled water, mix, and transfer to a plastic bottle for storage. Store in refrigerator until used.

b. Ammonium molybdate solution: Dissolve 20 g of $(NH_4)_6MO_7O_{24} \cdot 4H_2O$ in 500 mL of distilled water. Store in a plastic bottle at 4°C until used.

c. Ascorbic acid, 0.1 *M*: Dissolve 1.76 g of ascorbic acid in 100 mL of distilled water. The solution is stable for about a week if stored in an opaque plastic bottle at 4°C until used.

d. Potassium antimonyl tartrate solution: Using a 500 mL volumetric flask, dissolve 1.3715 g of $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ in approximately 400 mL of distilled water, and dilute to volume. Store in a dark, glass-stoppered bottle at 4°C until used.

e. Combined reagent: When making the combined reagent, all reagents must be allowed to reach room temperature before they are mixed, and they must be mixed in the following order. To make 100 mL of the combined reagent:

   i. Transfer 50 mL of 2.5 *M* H_2SO_4 to a plastic bottle.
   ii. Add 15 mL of ammonium molybdate solution to the bottle and mix.
   iii. Add 30 mL of ascorbic acid solution to the bottle and mix.
   iv. Add 5 mL of potassium antimonyl tartrate solution to the bottle and mix.

f. If turbidity has formed in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. Store in an opaque plastic bottle. The combined reagent is stable for less than 8 h, so it must be freshly prepared for each run.

g. Stock phosphate solution: Using a 1000 mL volumetric flask, dissolve 219.5 mg anhydrous KH_2PO_4 in distilled water and dilute to 1000 mL volume; 1 mL contains 50 μg of P.

h. Standard P solutions: Prepare a series of at least six standard P solutions within the desired P range by diluting stock phosphate solution with distilled water.

14.2.2 Procedure

1 Weigh out 2 g of air-dried soil into a 40 mL centrifuge tube. Conduct in duplicate.

2 Add 20 mL of distilled water and shake at 10 rpm end-over-end for 1 h.

3 Centrifuge at about 3000 *g* for 10 min.

4 Filter the solution through a Whatman No. 42 filter paper or 0.45 μm membrane filter if paper filtrates are not clear.

5 Measure P by ICP-AES or by the ascorbic acid technique (see Section 14.2.1).

6 Pipette 20 mL of water extraction filtrate into a 25 mL volumetric flask and add 5 mL of combined Murphy and Riley color reagent.

7 If the P concentration of the extract is greater than the highest standard, a smaller sample aliquot is required. Add revised sample aliquot to volumetric flask, make up to 20 mL with distilled water, and add Murphy and Riley reagent.

8 Measure absorbance (880 nm) and determine concentration from standard curve prepared each day.

14.2.3 Calculations

1 Water-extractable soil P (mg P kg soil^{-1})

$$= [\text{Concentration of P in extract, mg L}^{-1}] \times [\text{volume of extractant, L/mass of soil, kg}] \quad (14.1)$$

2 Minimum detection limit is 0.02 mg kg^{-1}.

3 There is no upper limit of detection, as extracts from soils with large amounts of P can be diluted.

14.2.4 Comments

Air-dried soils can be stored at room temperature in whirl-pack or closed plastic containers, to avoid contamination. Water extracts of soils should be kept at 4°C until P is measured, preferably within 2 days of extraction. A large amount of soil common to the users' area and similar to that being analyzed should be air-dried and archived. The water-extractable soil P concentration of the archived sample is run each day to ensure day-to-day analytical reproducibility. Any deviations form this standard value should be addressed immediately.

14.3 P SORPTION SATURATION

Phosphorus sorption saturation provides insight into a soil's ability to release P to solution as well as its remaining capacity to sorb added P and is defined as follows:

$$P_{sat} = \frac{\text{Sorbed P}}{\text{P sorption capacity}} \tag{14.2}$$

In the method described below, sorbed P is represented by Mehlich-3-extractable soil P and P sorption capacity by Mehlich-3-extractable Al and Fe. Notably, in estimating P sorption saturation from Mehlich data, this study does not include α, the proportion of Mehlich-3 Al and Fe that contribute to P sorption capacity. Use of α in the literature has been primarily associated with P sorption saturation calculated from acid ammonium oxalate data (e.g., van der Zee and van Riemsdijk 1988). Given soil-specific variations in sorption mechanisms affecting P sorption capacity as well as variability in methods used to estimate P sorption, there is little justification for the continued use of this value unless it is measured (Hooda et al. 2000).

14.3.1 Materials and Reagents

1 Centrifuge tubes (40 mL) with screw caps.

2 End-over-end shaker.

3 Centrifuge.

4 Filtration apparatus (0.45 μm pore diameter membrane filter or Whatman No. 42).

5 Mehlich-3 solution as 0.2 *M* CH_3COOH, 0.25 *M* NH_4NO_3, 0.015 *M* NH_4F, 0.013 *M* HNO_3, and 0.001 *M* EDTA (see Chapter 7 for more detail). Store in refrigerator until used.

6 Acid-washed glassware and plastic bottles.

14.3.2 Procedure

1 Weigh out 2.5 g of air-dried soil into a 40 mL centrifuge tube. Conduct in duplicate.

2 Add 25 mL of Mehlich-3 reagent and shake at 10 rpm for 5 min.

3 Filter the solution through a Whatman No. 42 filter paper or 0.45 μm membrane filter if paper filtrates are not clear.

4 Measure P, Al, and Fe by ICP-AES. Represented as P_{M3}, Al_{M3}, and Fe_{M3}, respectively.

14.3.3 Calculations

1 In all cases, molar concentrations of extracted elements (mmol kg^{-1}) were used to determine P_{sat}.

2 For acid soils (pH $<$ 7.0):

$$P_{sat} = \frac{P_{M3}}{Al_{M3} + Fe_{M3}} \tag{14.3}$$

14.3.4 Comments

Soil P sorption saturation is increasingly used as an environmental indicator of soil P availability to runoff and can be easily calculated from data that is readily available through soil testing laboratories and national databases. Several studies show that Mehlich-3 data can be effectively used to estimate P_{sat} for a wide range of acidic and alkaline soils. As most soil testing laboratories currently conducting Mehlich-3 extraction employ ICPs, analytes required to estimate P_{sat} (P_{M3}, Al_{M3}, and Fe_{M3}) are measured simultaneously. However, P estimated by ICP is often greater than by colorimetric methods due to ICP measuring near total (inorganic + organic) dissolved P. Care must be taken in building databases or comparing studies, which have used different methods of determining P in filtrates.

14.4 P SORPTION CAPACITY

Estimates of P sorption vary with soil/solution ratio, ionic strength and cation species of the supporting electrolyte, time of equilibration, range of initial P concentrations, volume of soil suspension to headspace volume in the equilibration tube, rate and type of shaking, and type and extent of solid/solution separation after equilibration (Nair et al. 1984). Even though a similar basic procedure is used to measure P sorption, there is considerable variation in the above parameters, which makes comparison of results among studies difficult. Thus, Nair et al. (1984) proposed a standard P adsorption procedure that would produce consistent results over a wide range of soils. This procedure was evaluated, revised, tested among laboratories, and was eventually proposed as a standardized P adsorption procedure and is detailed below.

14.4.1 Materials and Reagents

1 Centrifuge tubes (40 mL) with screw caps.

2 End-over-end shaker.

3 Centrifuge.

4 Filtration apparatus (0.45 μm pore diameter membrane filter or Whatman No. 42).

5 Photometer: Spectrophotometer with infrared phototube for use at 880 nm and providing a light path of at least 2.5 cm, preferably a 5 cm path length cell. For light path lengths of 0.5, 1.0, and 5.0 cm, the P ranges are 0.3–2.0, 0.15–1.30, and 0.01–0.25 mg L^{-1}, respectively.

6 Acid-washed glassware and plastic bottles: Graduated cylinders (5 to 100 mL), volumetric flasks (100, 500, and 1000 mL), storage bottles, pipets, dropper bottles, and test tubes or flasks for reading sample absorbance. The spectrophotometer should be calibrated daily using factory standard procedures for the laboratory machine.

7 Balances used to weigh reagents and samples are calibrated according to factory specifications and routinely cleaned to ensure proper and accurate working order.

8 Support or equilibrating solution is 0.01 *M* $CaCl_2$. Store in refrigerator until used.

9 Inorganic P solution of 50 mg L^{-1} as KH_2PO_4 in 0.01 *M* $CaCl_2$. Store in refrigerator until used.

14.4.2 Procedure

1 Weigh out 1 g of air-dried soil into a 40 mL centrifuge tube. Conduct in duplicate.

2 Add 0, 1, 2, 5, 10, 15, and 20 mL of stock P solution (50 mg L^{-1}) and make up to a final volume of 25 mL with distilled water and shake at 10 rpm end-over-end for 24 h. This gives equilibrating P concentrations of 0, 50, 100, 250, 500, 750, and 1000 mg P kg $soil^{-1}$ or 0, 2, 4, 10, 20, 30, and 40 mg P L^{-1}, respectively. The range of P concentrations used can be adjusted as needed to ensure the upper concentration represents a distinct curvature of the plotted P sorption isotherm.

3 Centrifuge at 3000 *g* for 10 min.

4 Filter the solution through a Whatman No. 42 filter paper or 0.45 μm membrane filter if paper filtrates are not clear.

5 Measure P by ICP-AES or by the ascorbic acid technique (see Section 14.2.1).

6 Pipette 5 mL of water extraction filtrate into a 25 mL volumetric flask and add 5 mL of combined Murphy and Riley color reagent and make up to 25 mL with distilled water.

7 Adjust sample aliquot as required and make up to a final volume of 25 mL after addition of Murphy and Riley reagent.

8 Measure absorbance (880 nm) and determine concentration from standard curve prepared each day.

14.4.3 Calculation of P Sorption Isotherm

1 The amount of P sorbed by soil (*S*, mg P kg $soil^{-1}$) is calculated as the difference between added P and P remaining in solution after the 24 h equilibration. Several methods exist for the determination of the amount of P originally sorbed by soil (S_o) such as the least squares fit model, oxalate-extractable P, and anion-membrane exchangeable P (Nair et al. 1998).

2 The Langmuir sorption isotherm is plotted as equilibrium solution P concentration (*C*, mg P L^{-1}) against P sorbed (*S*) as shown in Figure 14.1a.

3 Using the Langmuir sorption equation below, P sorption maximum (S_{max}, mg P kg $soil^{-1}$) and binding energy of P to soil (*k*, L mg P^{-1}) can be calculated.

$$\frac{C}{S} = \frac{1}{kS_{max}} + \frac{C}{S_{max}} \tag{14.4}$$

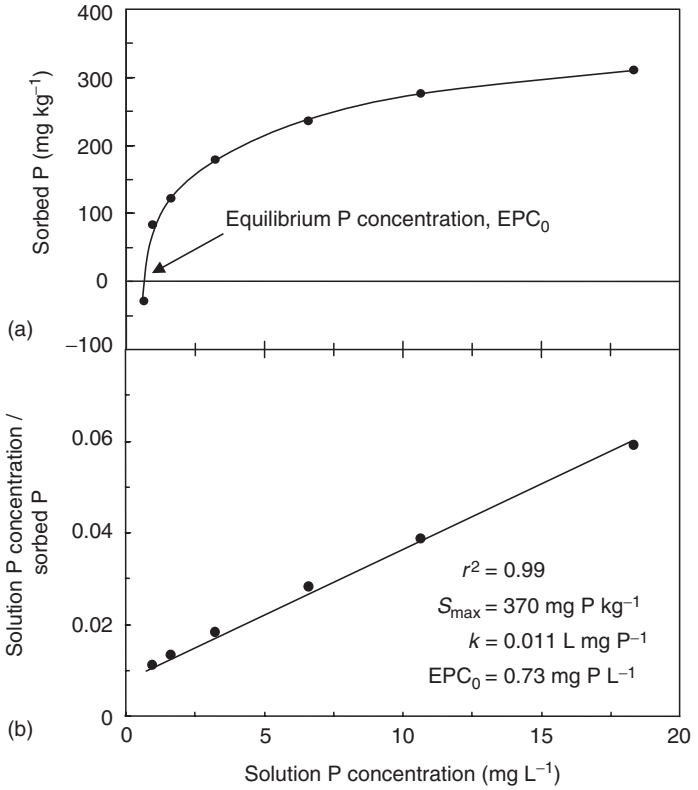

FIGURE 14.1. Representation of Langmuir P sorption isotherm (a) and linear (b) plot from which P sorption maximum, binding energy, and equilibrium P concentration are calculated.

where $S = S' + S_o$, the total amount of P sorbed (mg P kg soil^{-1}); S', P sorbed by soil (mg P kg soil^{-1}); S_o, P originally sorbed (previously sorbed P) (mg P kg soil^{-1}); C, equilibrium solution P concentration after 24 h shaking (mg P L^{-1}); S_{max}, P sorption maximum (mg P kg soil^{-1}); and k, a constant relating the binding energy of P to soil (L mg P^{-1}).

4 P sorption maximum, S_{max}, is calculated as the reciprocal of the slope of the plot C/S vs. C (Figure 14.1a).

5 Binding energy, k, is calculated as the slope/intercept of the same plot (Figure 14.1b).

6 The equilibrium P concentration (EPC_0, mg P L^{-1}), defined as the solution P concentration supported by a soil sample at which no net sorption or desorption occurs, is calculated as the intercept of the isotherm curve on the x-axis (see Figure 14.1).

14.5 P SORPTION INDEX

The procedure to determine PSI using a single-point isotherm approach, described below, is based on Bache and Williams (1971).

14.5.1 Materials and Reagents

1 Centrifuge tubes (40 mL) with screw caps.

2 End-over-end shaker.

3 Centrifuge.

4 Filtration apparatus (0.45 μm pore diameter membrane filter or Whatman No. 42).

5 Photometer: Spectrophotometer with infrared phototube for use at 880 nm and providing a light path of at least 2.5 cm, preferably a 5 cm path length cell. For light path lengths of 0.5, 1.0, and 5.0 cm, the P ranges are 0.3–2.0, 0.15–1.30, and 0.01–0.25 mg L^{-1}, respectively.

6 Acid-washed glassware and plastic bottles: Graduated cylinders (5 to 100 mL), volumetric flasks (100, 500, and 1000 mL), storage bottles, pipets, dropper bottles, and test tubes or flasks for reading sample absorbance. The spectrophotometer should be calibrated daily using factory standard procedures for the laboratory machine.

7 Balances used to weigh reagents and samples are calibrated according to factory specifications and routinely cleaned to ensure proper and accurate working order.

8 Inorganic P solution of 75 mg L^{-1} as KH_2PO_4 in 0.01 *M* $CaCl_2$. Store in refrigerator until used.

14.5.2 Procedure

1 Weigh out 1 g of air-dried soil into a 40 mL centrifuge tube. Conduct in duplicate.

2 Add 20 mL of the 75 mg P L^{-1} sorption solution to the centrifuge tube. This provides a single addition of 1.5 g P kg $soil^{-1}$ and a solution:soil ratio of 20:1.

3 Shake at 10 rpm end-over-end for 18 h.

4 Centrifuge at 3000 *g* for 10 min.

5 Filter the solution through a Whatman No. 42 filter paper or 0.45 μm membrane filter if paper filtrates are not clear.

6 Measure P by ICP-AES or by the ascorbic acid technique (see Section 14.2.1).

7 Pipette 5 mL of water extraction filtrate into a 25 mL volumetric flask and add 5 mL of combined Murphy and Riley color reagent and make up to 25 mL with distilled water.

8 Adjust sample aliquot as required and make up to a final volume of 25 mL after addition of Murphy and Riley reagent.

9 Measure absorbance (880 nm) and determine concentration from standard curve prepared each day.

14.5.3 Calculation of P Sorption Index

1 The PSI is calculated using the quotient $S/\log C$, where S is the amount of P sorbed (mg P kg^{-1}) and C is solution P concentration (mg L^{-1}).

2 Others have shown that expressing PSI directly as the amount of P sorbed (mg P kg^{-1}) is acceptable (Sims 2000).

14.6 P INDEX: SITE RISK ASSESSMENT FOR P LOSS VULNERABILITY

Site vulnerability to P loss in runoff is assessed with the P index by selecting rating values for a variety of source and transport factors. Although procedures and formats of P indices vary regionally, generally the first step in the process is to collect farm information such as farm maps, soil test reports, manure analysis, crop rotations, and manure handling and application information. The second step is to determine erosion rates, runoff class, and distance to receiving water is often needed. A site visit and evaluation is critical to properly evaluate field boundaries, areas of runoff and erosion contributions, and options for improved nutrient management and best management practices. The following procedure outlines the sources of information and calculations for Pennsylvania's P index. English units are most commonly used in P indices, to be consistent with the units used by field practitioners. Factors are given to convert English to metric or SI units.

The screening tool reduces potential time and workload associated with P index evaluations, by identifying fields at greatest risk to P loss using one or more readily available P index factors. In the Pennsylvania P index, the screening tool is Part A of the P index and uses soil test P (Mehlich-3 ppm P) and distance from the bottom edge of a field to a receiving body of water (contributing distance) (Table 14.1).

14.6.1 Procedure

If a soil test P level for a field is either greater than 200 ppm P or if the bottom edge of the field is closer than 150 ft. (50 m) to a receiving body, then the field is determined to have a potentially high risk of P loss. To determine the risk of P loss, additional field factors must be evaluated using Part B of the P index (Table 14.2).

If the soil test P level for the field is less than 200 ppm P and the bottom edge of the field is more than 150 ft. (50 m) from a receiving body, then the field does not have a potentially high risk for P loss and N-based nutrient management recommendations can be followed.

TABLE 14.1 The P Indexing Approach Using a Modified Version of Pennsylvania's Index Version of 8/2002, as an Example Part A—Screening Tool

	Evaluation category	
Soil test P—Mehlich-3 P	>200 ppm (μg g^{-1})	If yes to either factor
Contributing distance	<150 ft. (50 m)	then proceed to Part B

TABLE 14.2 The Transport Factors Included in Part B of the Pennsylvania P Index Version of 8/2002; Part B—Transport Factors

Characteristics	Risk levels					Risk value
Soil Erosion	Risk value = Annual soil loss = _____ tons/acre/year[a]					
Runoff Potential	Very Low **0**	Low **1**	Medium **2**	High **4**	Very High **8**	
Subsurface Drainage	None **0**		Random **1**		Patterned[b] **2**	
Leaching Potential	Low **0**		Medium **2**	High **4**		
Contributing Distance	>500 ft. (>150 m) **0**	500 to 350 ft. (100 to 150 m) **1**	350 to 250 ft. (75 to 100 m) **2**	150 to 250 ft. (50 to 75 m) **4**	<150 ft. (<50 m) **8**	
Transport Sum = Erosion + Runoff Potential + Subsurface Drainage + Leaching Potential + Contributing Distance						
Modified Connectivity	Riparian buffer *Applies to distances <150 ft. (<50 m)* **0.7**		Grassed waterway or None **1.0**	Direct connection *Applies to distances >150 ft. (>50 m)* **1.1**		
Transport Factor = Transport Sum × Modified Connectivity/22						

The transport value is divided by 22 (i.e. the highest value obtainable) in order to normalize site transport to a value of 1, where full transport potential is realized.

Caution: Many states in the United States have a state-specific P index. Although the principles of most P index tools are similar, individual factors or weightings of those factors vary among states. If available, review your own state's P index. For more specific information on the various indices adopted by states see Sharpley et al. (2003).

[a] 1 ton/acre/year is equivalent to 2.24 Mg ha^{-1} $year^{-1}$.
[b] Or a rapidly permeable soil near a stream.

14.6.2 Warning

Phosphorus indices vary with respect to factors evaluated, coefficients assigned to field conditions and management scenarios, and calculations used to determine P index values. Additionally, P indices are subject to change and modification to reflect current research and policy. In order to ensure the P index is being used and interpreted properly, current regionally approved versions must be obtained and regional training and certification requirements must be met by those specialists using the P index. The information that follows is based on Pennsylvania's P index.

14.6.3 Materials

1. Soil erosion: Soil erosion rate can be calculated using RUSLE 1.06 c (Renard et al. 1997).

2. Runoff potential: Using the predominate soil type in a field (50% or greater of the field area) and county specific tables, which can be provided by USDA-NRCS staff, the index surface runoff class can be determined for each evaluated field. The following describes the USDA-NRCS method for determining index surface runoff class.

3. Subsurface drainage: Using farm information, determine if there is artificial drainage in the field or if the field is near a stream and has rapidly permeable soils. ''Random'' drainage is a single or a few tile lines in a field and ''patterned'' drainage is when most or the entire field is drained with a fill-patterned drainage system. Rapidly permeable soils must occur within 150 ft. (50 m) of a stream and be classified as such by USDA-NRCS.

4. Contributing distance: Determine the contributing distance of each field to be evaluated to receiving water. The distance is measured from the lower edge of the field closest to the receiving water and can be determined using farm maps or by field measurements.

5. Modified connectivity: Accounts for if and where buffers, grassed waterways, ditches, and pipe outlets are present.

    - If the field is within 150 ft. (50 m) of water and a riparian buffer is present, select the appropriate modified connectivity factor (i.e., reduces transport value). All buffers must be designed and maintained to meet USDA-NRCS standards.

    - If a field is more than 150 ft. (50 m) from water but a direct connection such as a pipe or ditch from field to receiving water is present, select appropriate modified connectivity factor (i.e., increases transport value).

    - If a field has a grassed waterway or has no qualifying management practices, then a default coefficient of 1.0 is used (i.e., the transport value is neither increased nor is it decreased).

14.6.4 Calculations

1. Transport sum: Sum the actual soil loss rate (tons/acre/year) with the coefficients for runoff potential, subsurface drainage, and contributing distance. Enter the sum into the transport sum risk value column for each field.

2. Transport factor: Multiply the transport sum by the modified connectivity coefficient and divide the product by 22. Twenty-two is the maximum transport sum value and dividing by this value allows the transport factor to vary generally between 0 and 1. One is the value at which the full (100%) field transport potential is reached. Any other value would represent a relative percentage of the field's full transport potential. The transport factor only exceeds 1 when erosion losses are exceptionally high. Enter the product into the transport factor risk value column for each field.

14.6.5 Materials

1. Soil test P: Current soil test reports.

2. Fertilizer and manure rate: Farm records or a nutrient management plan indicating the amount of P, in pounds of P_2O_5/acre, to be applied to each field.

3. Loss rating for fertilizer and manure application: Farm records or a nutrient management plan indicating the methods and timing used to apply P to each field.

4. Manure P availability: Farm records or a nutrient management plan indicating the manure types, manure groups, or other organic P sources to be applied to each field to be evaluated (Table 14.3).

14.6.6 Calculating the P Index Value

1. Enter all of the transport factors (Part B) and sums of management factors (Part C) into the worksheet below.

2. Multiply Part B by Part C and then the product by 2. The factor of 2 normalizes the final index rating to 100. This is your final P index rating.

3. Look up the associated interpretation and management guidance in Table 14.4.

Field	Part B transport risk	Part C management risk	P Index B × C × 2	Interpretation of the P index
Example	0.55	92	101	Very high

TABLE 14.3 Phosphorus Loss Potential due to Source and Site Management Factors in the P Index; Part C—Source and Site Factors

Contributing Factors	Risk Levels					Risk Value
	Very Low	**Low**	**Medium**	**High**	**Very High**	
Soil test P risk[a]	Risk Value = Soil Test P (ppm as Mehlich-3 P) × 0.20 = _____ ppm × 0.20 = **OR** Risk value = Soil test P (lbs P_2O_5/acre) × 0.05 = _____ lbs P_2O_5/acre × 0.05 =					
Loss rating for P application method and timing	Placed with planter or injected more than 2" (5 cm) deep **0.2**	Incorporated <1 week after application **0.4**	Incorporated >1 week or not incorporated >1 following application in spring-summer **0.6**	Incorporated >1 week or not incorporated following application in autumn-winter **0.8**	Surface applied on frozen or snow covered soil **1.0**	
Fertilizer P risk[a]	Risk Value = Fertilizer P Application Rate × Loss Rating for P Application = Risk Value = _____ lbs P_2O_5/acre × _____ =					
Manure P availability	Organic Phosphorus Source Availability Coefficients					
Manure P risk[a]	Risk Value = Manure P Application Rate × Loss Rating for P Application × P Availability Coefficient = Risk Value = _____ lbs P_2O_5/acre × _____ × _____ =					
Total of Management Risk Factors				**Sum of management factors**	**=**	

Caution: Many states in the United States have a state-specific P index. Although the principles of most P index tools are similar, individual factors or weightings of those factors vary among states. If available, review your own state's P index. For more specific information on the various indices adopted by states see Sharpley et al. (2003).

[a] Conversion factor: 10 lbs P_2O_5/acre is equivalent to 4.89 kg P ha^{-1}.

TABLE 14.4 General Interpretations and Management Guidance for the P Index

P Index value	Rating	General interpretation	Management guidance
<59	Low	If current farming practices are maintained, there is a low risk of adverse impacts on surface waters	N-based applications
60–79	Medium	Chance for adverse impacts on surface waters exists, and some remediation should be taken to minimize P loss	N-based applications
80–100	High	Adverse impact on surface waters. Conservation measures and P management plan are needed to minimize P loss	P application limited to crop removal of P
>100	Very high	Adverse impact on surface waters. All necessary conservation measures and P management plan must be implemented to minimize P loss	No P applied

Caution: Many states in the United States have a state-specific P index. Although the principles of most P index tools are similar, individual factors or weightings of those factors vary among states. If available, review your own region's P index. For more specific information on the various indices adopted by states see Sharpley et al. (2003).

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Chapter 15
Electrical Conductivity and Soluble Ions

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15.1 INTRODUCTION

Saline soil is defined as one containing sufficient soluble salts to adversely affect the growth of most crop plants (Soil Science Society of America 2001). Soil salinization is a widespread limitation to agricultural production in dryland and irrigated soils throughout the world. Soil salinity reduces crop growth because depression of the osmotic potential of the soil solution limits water uptake by the plant (Corwin and Lesch 2003). Salinity may also cause specific ion toxicity or nutrient imbalances, and soil permeability and tilth may deteriorate if excessive amounts of Na accumulate on the soil's cation-exchange complex.

Soil salinity is generally measured by the electrical conductivity (EC) of a soil extract. A soil is considered saline if the EC of the saturation extract exceeds 4 dS m^{-1} at 25°C (Soil Science Society of America 2001). The main ions comprising soluble salts are cations Na, Ca, Mg; and anions SO_4, and Cl. Minor amounts of K, HCO_3, CO_3, and NO_3 may also be present. Soil sodicity is the accumulation of exchangeable Na, determined by measuring the exchangeable sodium percentage (ESP); or more commonly, estimated from the sodium adsorption ratio (SAR) of a soil–water extract. If the SAR of the saturation extract exceeds 13, the soil is considered sodic (Soil Science Society of America 2001). A more detailed classification scheme for sodic soils based on physical behavior (clay dispersibility), sodium, and salinity levels, has been proposed by Sumner et al. (1998).

Soil salinity or EC may be measured on the bulk soil (EC_a), in the saturation paste extract (EC_e), in water extracts at soil:water ratios of 1:1 to 1:5 ($EC_{1:1}$, $EC_{1:2}$, $EC_{1:5}$), or directly on soil water extracted from the soil in the field (EC_w) (Corwin and Lesch 2003). The EC_a or apparent EC has become one of the most reliable and frequently used measurements to characterize the spatial distribution of soil salinity at field scales. Field methods used to

measure EC_a include Wenner array or four-electrode, electromagnetic (EM) induction, and time domain reflectometry (TDR) (Rhoades and Oster 1986; Rhoades 1990, 1992). The EM induction method is the most popular of these three methods because measurements can be taken quickly over large areas, the large volume of soil measured reduces local-scale variability, and measurements are possible in relatively dry or stony soils because no contact is necessary between the soil and EM sensor (Hendrickx et al. 1992). The EM38 meter, and to a lesser extent, the EM31 meter (Geonics Ltd., Mississauga, Ontario) are most commonly used in soil investigations. The EM38 can measure EC_a to a depth of 1.2 m in the vertical mode and to 0.6 m in the horizontal mode. Mobile systems have been developed in conjunction with global positioning systems (GPS) to allow rapid salinity mapping of large fields (Rhoades 1992; Cannon et al. 1994). The EC_a readings from the EM38 meter are easily converted to EC_e values for different soil temperature, texture, and moisture conditions (Rhoades and Corwin 1981; Corwin and Rhoades 1982; McKenzie et al. 1989).

The EC of aqueous extracts of soil has traditionally been defined in terms of the EC of the saturated soil paste extract (EC_e) (U.S. Salinity Laboratory Staff 1954). Since the EC and concentration of solutes are affected by the soil:water ratio (Reitemeier 1946), this needs to be standardized to allow for consistent universal interpretation across soil texture classes. Exceptions include sandy soils, organic soils, and soils containing gypsum (Robbins and Wiegand 1990). Since it is impractical to routinely extract soil water at typical field-water contents, soil solution extracts must be made at higher than normal water contents. The saturated soil paste approximates the lowest soil:water ratio at which sufficient extract can be routinely removed for analysis of major salinity constituents. The saturated paste method relates more closely to the water holding capacity of the soil than do extracts at a fixed soil:water ratio. The water content of a saturation paste is about twice that at field capacity for most soils (Robbins and Wiegand 1990). Crop tolerance to salinity has traditionally been expressed in terms of EC_e.

Because the saturated paste method requires time and skill, laboratories are increasingly using fixed soil:water ratios (e.g., 1:1, 1:2, 1:5) when measuring soil EC and solute concentrations. However, cation exchange and mineral dissolution as the soil:water ratio widens (Reitemeier 1946) may lead to overestimation of EC and changes in solute composition. This is especially the case in samples containing gypsum, since Ca and SO_4 concentrations remain near-constant over a range of soil:water ratios while the concentration of the other ions decreases with dilution (Robbins and Wiegand 1990). Nevertheless, studies have shown good correlations between EC, Mg, K, and Cl in 1:2 extracts versus saturation paste extracts (Sonneveld and Van den Ende 1971); between EC, Na, Ca + Mg, and Cl in 1:1 and 1:2 extracts versus saturation paste extracts (Hogg and Henry 1984); and between EC, soluble cations (Na, Ca, Mg, K) and anions (SO_4, Cl) in 1:1 extracts versus saturation paste extracts (Pittman et al. 2004). In an analysis of soluble salt data from 87 laboratories in the United States, average residual standard deviation (RSD) was lowest for saturation paste extracts (13.4%), followed by 1:1 extracts (24.2%), and then 1:2 extracts (32.5%) (Wolf et al. 1996). Ninety percent of results for the 1:1 extracts were within ± 2 standard deviations of the mean value (acceptable laboratory performance) compared with 87% of the saturation paste extracts, and 84% of the 1:2 extracts.

Measurement of EC (EC_w) and solutes in the soil water extracted at field-water content is theoretically the best measure of salinity because it indicates the actual salinity level experienced by the plant root (Corwin and Lesch 2003). However, EC_w has not been widely used because it varies as soil–water content changes over time and so it is not a single-valued parameter (Rhoades 1978), and the methods for obtaining soil solutions are too laborious and

costly to be practical (Rhoades et al. 1999). Soil solutions can be obtained from disturbed samples by displacement, compaction, centrifugation, molecular adsorption, and vacuum or pressure extraction methods (Rhoades and Oster 1986). Soil solutions from undisturbed samples can be obtained using various suction-type samplers and salinity sensors (Corwin and Lesch 2003). Kohut and Dudas (1994) reported considerable variation between the properties of saturation paste extracts and immiscibly displaced solutions, with the saturation paste extract having lower EC values, cation concentrations (Na, Mg, K), and anion concentrations.

This chapter will focus mainly on laboratory methods used to measure EC of saturation paste extracts and extracts at fixed soil:water ratios, and on methods available to analyze soluble cations and anions in these extracts.

15.2 EXTRACTION

15.2.1 Saturation Extract (Janzen 1993; Rhoades 1996)

Procedure

1 Determine moisture content or weight of water in air-dry soil samples to be used. Weigh a subsample (30–50 g) of air-dry soil, oven-dry at 105°C, reweigh it, and determine weight of water in air-dry soil.

2 Weigh from 200 to 400 g of air-dry soil with known moisture content into a container with lid. Record the total weight of container and the soil sample. (The weight of soil used will depend on the volume of extract required. In general, approximately one-third of the water added is recovered in the saturation extract.)

3 Add deionized water while mixing to saturate the soil sample. At saturation, the soil paste glistens, flows slightly when the container is tipped, slides cleanly from the spatula, and readily consolidates after a trench is formed upon jarring the container.

4 Allow the sample to stand for at least 4 h and check to ensure saturation criteria are still met. If free water has accumulated on the surface, add a weighed amount of soil and remix. If the soil has stiffened or does not glisten, add distilled water and mix thoroughly.

5 Weigh the container with contents. Record the increase in weight, which corresponds to the amount of water added. (Alternatively, the amount of water added can be determined volumetrically by dispensing water from a burette.) Calculate the saturation percentage (SP) as follows:

$$SP = \frac{(\text{weight of water added} + \text{weight of water in sample})}{\text{oven-dry weight of soil}} \times 100 \qquad (15.1)$$

6 Allow the paste to stand long enough to establish equilibrium between the soil minerals and the water (at least 4 h, but preferably overnight). If a pH measurement is needed, the samples are then thoroughly mixed and their pH measured with an electrode and pH meter. The pH of the saturation paste is generally more meaningful than the pH of the saturation paste extract (Robbins and Wiegand 1990).

7 Transfer the wet soil to a Buchner funnel fitted with highly retentive filter paper. Apply vacuum and collect extract until air passes through the filter. Turbid filtrates should be refiltered.

8 Store extracts at 4°C until analyzed for EC and soluble cations and anions.

Comments

If possible, organic soils should be extracted without prior drying, which affects the SP. Organic soils may require an overnight saturation period and a second addition of water to achieve a definite saturation endpoint. For fine-textured soils, sufficient water should be added immediately to the soil sample with minimal mixing to bring the sample close to saturation. Do not over-wet coarse-textured soils. Free water on the soil surface after standing indicates oversaturation of coarse-textured soils.

15.2.2 Fixed Ratio Extracts (Janzen 1993; Rhoades 1996)

Procedure

1 Weigh appropriate amount of air-dry soil into a flask, add sufficient deionized water to achieve desired extraction ratio, and shake for 1 h.

2 Filter the suspension using highly retentive filter paper and store filtrate at 4°C before analysis.

Comments

The 1:1 and 1:2 soil:water extraction ratios are preferred over the 1:5 ratio. However, the 1:5 ratio is commonly used in Australian salinity work (Rengasamy et al. 1984; Sumner et al. 1998).

15.3 ANALYSES

15.3.1 Electrical Conductivity (EC_e, $EC_{1:1}$, $EC_{1:2}$, $EC_{1:5}$)

The total solute concentration in the various extracts is normally estimated by measuring EC. Although the relationship between conductivity and salt concentration varies somewhat depending on solution ionic composition, EC provides a rapid and reasonably accurate estimate of solute concentration. The procedure below is for modern EC meters that provide automatic temperature compensation, automatically adjust cell constant internal to the meter, and readout EC directly in μmho cm^{-1} or similar units. For older EC meters that do not have these three features, refer to Rhoades (1996) or American Public Health Association (1998).

Procedure

1 Make up standard 0.010 *M* KCl solution to automatically adjust cell constant internal to the meter. Dissolve 0.7456 g of reagent-grade anhydrous KCl and make up to 1 L using pure water (EC < 0.001 dS m^{-1}). This solution has an EC of 1.413 dS m^{-1} at 25°C and is suitable for most solutions when the cell constant is between 1 and 2. Use stronger or weaker KCl solutions to determine other cell constants.

2 Calibrate conductivity meter using standard KCl solution to automatically adjust cell constant internal to the meter. Rinse probe three times with 0.01 *M* KCl. Adjust temperature of a fourth portion to 25.0°C ± 0.1°C. Adjust temperature compensation dial to 0.0191 C^{-1}. With probe in standard KCl solution, adjust meter to read 1413 μmho cm^{-1} or 1.413 dS m^{-1}.

3 Read conductivity of extracts using EC probe and meter. Report results in SI units of dS m^{-1}.

Comments

Use an EC meter capable of measuring EC with an error not exceeding 1% or 1 μmho cm^{-1} or 0.001 dS m^{-1}. The basic unit of EC is mho cm^{-1}, and is too large for most natural waters (Bohn et al. 1979). A more convenient unit is mmho cm^{-1}. Units in the older literature, or when dealing with low salinity waters, have also been reported as μmho cm^{-1}. The SI unit of conductivity is siemens per meter (S m^{-1}), but results are generally reported as dS m^{-1}. Water with an EC of 0.0002 mho cm^{-1} has an EC of 0.2 mmho cm^{-1}, 200 μmho cm^{-1}, 0.020 S m^{-1}, or 0.2 dS m^{-1}.

15.3.2 Soluble Ion Concentrations—Overview and Comparison of Methods

Various methods are available to analyze soluble cations and anions in soil–water extracts (Table 15.1). Most laboratories have used flame-atomic absorption spectroscopy (FL-AAS) to analyze soluble cations, colorimetric methods on an autoanalyzer to determine Cl and SO_4, and the titrimetric method to analyze HCO_3 and CO_3.

FL-AAS is the preferred instrument for analyzing soluble cations where cost is a major limitation, number of samples will not be large, and extremely low detection limits are not required (Wright and Stuczynski 1996). Ion chromatography (IC) has been mostly used to analyze SO_4 and Cl in aqueous systems (American Public Health Association 1998) and soil extracts (Nieto and Frankenberger 1985a). Although IC can also determine soluble cations in soils (Basta and Tabatabai 1985; Nieto and Frankenberger 1985b), it is seldom used for cation analysis.

TABLE 15.1 Methods That Could Be Used to Measure Concentrations of Soluble Cations and Anions in Saturation Paste and Fixed Ratio Extracts

Method[a]	Na	K	Ca	Mg	Cl	SO_4	HCO_3/CO_3
FL-AAS	X	X	X	X			
IC	X	X	X	X	X	X	
ICP-AES	X	X	X	X	X		
Gravimetric					X	X	
Colorimetric					X	X	
Electrometric					X		X
Turbidimetric						X	
Titrimetric						X	X

[a] FL-AAS, flame-atomic absorption spectroscopy; IC, ion chromatography; ICP-AES, inductively coupled plasma-atomic emission spectroscopy.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) has been increasingly used to analyze soluble Na, K, Ca, and Mg in soil extracts (Soltanpour et al. 1996; Wright and Stuczynski 1996) and waters (Vitale et al. 1991). In addition, ICP-AES can be used to determine nonmetals such as S and Cl in aqueous extracts (Richter et al. 1999). The advantages of ICP-AES are the plasma flux is extremely stable compared to conventional flames with FL-AAS, lower detection limits are possible for certain elements, and it has simultaneous multielement capability where 15 to 20 metals in a water sample can be measured in a 2 min period (Vitale et al. 1991). Disadvantages with ICP-AES are high initial cost, high operating costs (gases, power, consumables) (Wright and Stuczynski 1996), and possible severe matrix interferences from high concentrations of total dissolved solids, Na, Ca, Fe, and Al (Vitale et al. 1991).

Soluble Cations

Sodium has been most commonly analyzed using flame emission photometry at 589 nm, and K using flame photometry at 766.5 nm (Robbins and Wiegand 1990; Helmke and Sparks 1996). Pretreatment involves filtering out any solid particles. Calcium has been traditionally analyzed using AAS at 422.7 and 285.2 nm, respectively (Robbins and Wiegand 1990; Suarez 1996). Elements that form stable oxysalts (Al, Be, P, Si, Ti, V, Zr) can interfere with Ca and Mg analyses, but these can be removed by adding 0.1% to 1.0% lanthanum or strontium chloride to the samples.

Soluble Anions

Chloride in soil extracts is most commonly analyzed using potentiometric titration with $AgNO_3$, direct potentiometric analysis using a solid-state selective ion-electrode, automated colorimetric analysis (mercury thiocyanate method) on the autoanalyzer, or by IC (Frankenberger et al. 1996). The mercury thiocyanate method is widely used to determine Cl, but there is a trend toward IC and ICP-AES methods to avoid working with, and disposing of, Hg and cyanate. Gravimetry, turbidimetry, titrimetry, and colorimetry are the most common methods to analyze SO_4 in soil extracts; but the most sensitive and accurate methods for soil extract analyses are the methylene blue (MB) colorimetric and IC methods (Tabatabai 1996). In addition, the automated methylthymol blue method on the autoanalyzer is commonly used to measure SO_4 in aqueous systems (American Public Health Association 1998). This method can directly measure SO_4 in water, unlike the MB colorimetric method, which requires reduction of SO_4 to H_2S. However, similar to Cl, some laboratories are increasingly using IC and ICP-AES to measure SO_4 to avoid working with, and disposing of, thymol. Carbonate and bicarbonate ions are most commonly determined by titrating (titrimetric method) samples to an endpoint of pH 8.4 using phenolphthalein (CO_3) and then to pH 4.7 using methyl orange (HCO_3) (U.S. Salinity Laboratory Staff 1954). Alternatively, a pH probe and meter (electrometric method) can be used to determine the endpoints.

15.4 CALCULATIONS AND INTERPRETATION

15.4.1 Electrical Conductivity

Salt tolerance data for crops have been developed relating crop yield to EC_e. Data have been compiled for 69 herbaceous crops based on controlled tests in the United States and India (Maas 1990) for soils where chloride salts predominate. Salt tolerance data have also been compiled by Ayers and Westcott (1985). Crops grown on gypsiferous soils, such as found in the Canadian Prairies, will tolerate an EC_e of about 2 dS m^{-1} higher than those listed in Maas's table. In Canada, salt tolerance data based on field tests at specific locations have been

TABLE 15.2 Crop Response to Salinity Measured as Electrical Conductivity (EC) of the Saturation Extract

EC (dS m^{-1} at 25°C)	Crop response
0–2	Almost negligible effects
2–4	Yields of very sensitive crops restricted
4–8	Yields of most crops restricted
8–16	Only tolerant crops yield satisfactorily
>16	Only very tolerant crops yield satisfactorily

Source: Adapted from Bernstein, L., *Ann. Rev. Phytopathol.*, 13, 295, 1975.

reported by Holm (1983) and McKenzie (1988). More recently, research at Canada's salt tolerance testing facility (Steppuhn and Wall 1999) reported salt tolerance data for spring-sown wheats (Steppuhn and Wall 1997), as well as for canola, field pea, dry bean, and durum wheat crops (Steppuhn et al. 2001). General salinity effects are presented in Table 15.2.

15.4.2 Expressing Results of Soluble Ion Analyses

Soluble salt data are generally expressed in units such as meq L^{-1} ($mmol_c$ L^{-1}), mg L^{-1}, or mmol L^{-1}. If the results are to be expressed on a mass basis (e.g., mg of Ca per kg of soil), then the mass of air-dry soil, the mass of water added, and water already in the soil need to be known.

15.4.3 Ion Activities and Saturation Index Values

Soil solution data are generally reported as ion concentrations. However, it may sometimes be desirable to express the results as ion activities or thermodynamically effective concentration. The activity of an element, rather than its concentration, may be more closely related to plant response (Adams 1966) and general chemical reactivity (Freeze and Cherry 1979). Ion activity is the product of the ion concentration and the activity coefficient. There is an inverse relationship between the activity coefficient and ionic strength of the soil solution. As salinity or ionic strength of the aqueous solution increases, the activity coefficient decreases, resulting in a lower ion activity that can participate in chemical reactions. Increasing salinity also increases the solubilities of minerals via the ionic strength effect. Ion activities can be estimated from various geochemical models, and some ion activities (e.g., Cl, K) can be directly measured in solution extracts using ion-selective electrodes. Saturation index (SI) values for minerals can also be estimated from geochemical models by dividing the ion activity product of the solution species composing the mineral of interest by the solubility product constant (K_{sp}) of the mineral. SI values <0 indicate undersaturation or dissolution with respect to the mineral, $SI = 0$ indicates saturation or equilibrium between the solution and solid phase, and $SI > 0$ indicates supersaturation or precipitation of the mineral. However, SI values for evaporate minerals from saline soils were found to be poor predictors of minerals formed in evaporated soil solutions (Kohut and Dudas 1994).

15.4.4 Sodium Adsorption Ratio

The SAR, a useful index of the sodicity or relative sodium status of soil solutions, and aqueous extracts, or water in equilibrium with soil, is calculated as follows:

$$SAR = \frac{[Na^+]}{[Ca^{2+} + Mg^{2+}]^{0.5}} \tag{15.2}$$

where cation concentrations are in mmol L^{-1}.

Soils with SAR values greater than 13 are considered to be sodic (Soil Science Society of America 2001), although other critical values have been proposed (Bennett 1988; Sumner et al. 1998). Equation 15.2 is often referred to as the practical SAR (SAR_p), whereas theoretical SAR (SAR_t) values are calculated using the same equation but with free ion activities instead of concentrations (Kohut and Dudas 1994). Since exchangeable cations are difficult to measure in saline soils because of errors arising from anion exclusion or dissolution of slightly soluble minerals, the SAR of soil aqueous extracts has become the principal tool for diagnosing sodic soils (Bohn et al. 1979; Jurinak 1990).

15.4.5 Exchangeable Sodium Percentage

ESP can be estimated from SAR based on the linear equation:

$$\frac{\text{ESP}}{[100 - \text{ESP}]} = K_g\text{SAR} \tag{15.3}$$

where K_g is the Gapon selectivity coefficient. The value of K_g has traditionally been taken as 0.015 $(\text{mmol L}^{-1})^{-0.5}$ (U.S. Salinity Laboratory Staff 1954), though K_g can vary depending on soil organic matter content and pH (Curtin et al. 1995). In general, the affinity of soils for Na decreases as the contribution of organic matter to the cation-exchange capacity increases.

15.4.6 Potassium Adsorption Ratio

The potassium adsorption ratio (PAR) is calculated by substituting K for Na in Equation 15.2. Excessive K concentrations may interfere with crop uptake of other nutrients, decrease soil hydraulic conductivity and permeability, and increase soil erodibility (Hao and Chang 2003). Potassium concentrations are high in livestock manures, and K may become the dominant soluble cation in manured soils (Pratt 1984). Pratt (1984) reported that the long-term hazard of the use of manures on well-leached irrigated lands was more from K than from Na accumulation. Critical PAR values to define soils with excessive K remain to be determined.

15.4.7 Critical Calcium Ratio

A number of studies have shown that crop yield in a salt-affected soil is strongly influenced by the ratio of Ca to that of other cations in the soil solution (Howard and Adams 1965; Carter et al. 1979; Janzen and Chang 1987; Janzen 1993). Yield reductions are typically observed when the ratio of Ca:total cations is below approximately 0.10. This ratio can fall below the critical value in sodic soils (Carter et al. 1979) and in saline, gypsiferous soils where Ca concentrations are low because of the poor solubility of $CaSO_4 \cdot 2H_2O$ (gypsum) (Curtin et al. 1993).

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