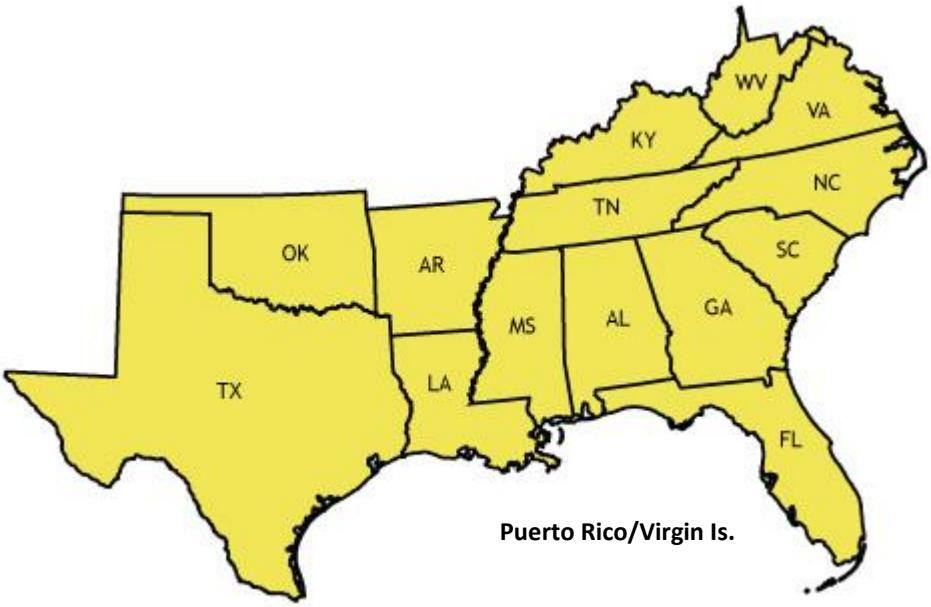
# Soil Test Methods
# From the Southeastern United States

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Southern Extension and Research Activity Information Exchange Group - 6
(SERA-IEG-6)

Edited by:
F.J. Sikora
Division of Regulatory Services
University of Kentucky
Lexington, KY 40546

K.P. Moore
Agricultural Service Laboratory
Clemson University
Clemson, SC 29634

**Authors:**
K.K. Crouse
D.H. Hardy
S. Heckendorn
G. Huluka
D.K. Joines
D.E. Kissel
R. Miller
C.C. Mitchell
K.P. Moore
R. Mylavarapu
J.L. Oldham
T. Provin
H.J. Savoy
F.J. Sikora
L. Sonon
J.J. Wang
D.D. Warncke
H. Zhang

**Editorial Committee:**
F.J. Sikora
K.P. Moore
G. Huluka
D.E. Kissel
R.Miller
R. Mylavarapu
J.L. Oldham

A current list of the members, participants, and administrative advisors of SERA-IEG-6 may be found at www.clemson.edu/sera6

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# Author Contact Information

Crouse, Keith K. Soil Testing Laboratory, Box 9610, Mississippi State, MS 39762. Phone: 662-325-3313. Email: keithc@ext.msstate.edu

Hardy, David H. NCDA & CS Agronomic Division, Soil Testing Section, 4300 Reedy Creek Road, 1040 Mail Service Center, Raleigh, NC 27699. Phone: 919-733-2837. Email: david.hardy@ncagr.gov

Heckendorn, Steve. Virginia Tech Soil Testing Lab, 145 Smyth Hall (0465), Blacksburg, VA 24061. Phone: 540-231-9807. Email: soiltesting@vt.edu

Huluka, Gobi. Department of Agronomy & Soils, 201 Funchess Hall, Auburn University, AL 36849. Phone: 334-844-3926. Email: hulukgo@auburn.edu

Joines, Debbie K. University of Tennessee, Soil, Plant, and Pest Center, 5201 Marchant Drive, Nashville, TN 37211. Phone: 615-832-4936. Email: Djoines@utk.edu

Kissel, David E. Ag and Environmental Services Lab, University of Georgia, 2400 College Station Rd, Athens, GA 30602. Phone: 706-542-5350. Email: dkissel@uga.edu

Miller, Robert . ALP & MAP Programs, Colorado State University, Fort Collins, CO 80523. Phone: 970-686-5782. Email: rmiller@colostate.edu

Mitchell, Charles C. Department of Agronomy and Soils, 201 Funchess Hall, Auburn University, AL 36849. Phone: 334-844-5489. Email: mitchc1@auburn.edu

Moore, Kathy P. Agricultural Service Laboratory, Clemson University, 171 Old Cherry Road, Clemson, SC 29634. Phone: 864-656-2300. Email: kmr@clemson.edu

Mylavarapu, Rao. Soil & Water Science Department, University of Florida, 171 McCarty Hall, P. O. Box 110290, Gainesville, FL 32611. Phone: 352-294-3113. Email: raom@ufl.edu

Oldham, J. Larry. Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. Phone: 662-325-2760. Email: loldham@pss.msstate.edu

Provin, Tony. 2478 Texas A&M University, Texas Agri Life Extension Service, Soil & Crop Science Department, College Station, TX 77843. Phone: 979-862-4955. Email: t-provin@tamu.edu

Savoy, Hugh J. University of Tennessee, Room 212 Biosystems Engineering and Soil Science Building, 2506 E. J. Chapman Drive, Knoxville, TN 37796. Phone: 865-974-8840. Email: hsavoy@utk.edu

Sikora, Frank J. University of Kentucky, Soil Testing Lab, 103 Regulatory Service Building, Lexington, KY 40546. Phone: 859-218-2452. Email: fsikora@uky.edu

Sonon, Leticia. Soil, Plant, and Water Laboratory, University of Georgia, 2400 College Station Road, Athens, GA 30602. Phone: 706-542-5350. Email: lsonon@uga.edu

Wang, Jim J. Louisiana State University, School of Plant, Environmental and Soil Sciences, 313 Sturgis Hall, Baton Rouge, LA 70803. Phone: 225-578-1360. Email: jjwang@agctr.lus.edu

Warncke, Darryl D. Michigan State University, Crop and Soil Sciences Dept., East Lansing, MI.

Zhang, Hailin. Oklahoma State University, Department of Plant and Soil Sciences, 368 Agriculture Hall, Stillwater, OK 74078. Phone: 405-744-9566. Email: hailin.zhang@okstate.edu

# Foreword

The Southern Extension and Research Activity Information Exchange Group (SERA-IEG-6) was formed in 1954 under the name of Southern Region Soil-Testing Work Group. The justification for forming the Group was to reconcile differences in fertilizer recommendations across state lines. Before reconciling differences in recommendations, differences in soil test methods amongst the states had to be assessed. The Group published their first methods publication in 1965 (Page, 1965). The publication documents 13 different soil extractants for phosphorus, potassium, calcium, and magnesium used amongst University laboratories in the Southeastern states. An objective of the publication was to present detailed methodology so methods can be exchanged and studied to determine the best ones to use.

In 1983, the Group published an updated methods publication with 9 standard reference procedures (Donohue and Isaac, 1983). Progress was made on encouraging standardization and uniformity in soil test methods with only 3 soil extractants for phosphorus, potassium, calcium, and magnesium presented in the 1983 publication compared to 13 extractants in the 1965 publication. The 3 extractants included Mehlich 1 for P, Bray for P, and ammonium acetate for K, Ca, and Mg. The 1983 publication was limited as it only presented methods for the plant nutrients phosphorus, potassium, calcium, and magnesium. More soil test methods were published by the Group in 1992 which included Mehlich-3 and methods for sulfate-sulfur, nitrate-nitrogen, and micronutrients (Donohue, 1992). The publication also included additional methods to assess soil acidity and organic matter and to test strip mined soil, potting media, and waste amended soil.

Several advancements in instrument technology and methods have occurred since the 1992 methods publication. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is now commonly used for the determination of a wide range of plant nutrients in soil extracts, including phosphorus which was formerly determined via spectrophotometric analysis. Robotic pH instruments have become widely available for determining soil and buffer pH for determining lime requirement. Several methods have been developed to quantify soil acidity using nonhazardous reagents. Combustion instruments are now commonplace for measurement of C and N in soil. These advancements necessitated an update to the 1992 methods publication. The objectives of the current publication were to document the methods accepted and used at University laboratories in the Southern Region (Savoy, 2013), document the progress the Southern Region has made in advancing soil test methodology, help laboratories readily adopt methods, and provide information for further research and development.

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# *Unit 1*

# Soils

*Chapter 1.1*

# Soils of the Southeastern US

*R. Mylavarapu, C.C. Mitchell, and H.J. Savoy*

The US states and territories that participate in the Southern Extension and Research Activities Information Exchange Group (SERA-IEG-6) have a diversity of geography and climate that influence the fertility and productivity of their soils (Buol, 1973). Discounting Texas, the states contain four of the eight physiographic regions in the US (Fig. 1). Soils within these regions have similar origins and often have comparable physical and chemical characteristics. The various soil orders follow a pattern similar to the physiographic regions (Fig. 2).

The formation of soils is a complex process of weathering and is a dynamic and combined function of parent material, climate, topography, biology and time. Soils of the Southeastern US were generally developed with abundant rainfall under forests. The abundant rainfall leached base cations, such as potassium, calcium, magnesium, and sodium, which has resulted in soils having an acidic nature. The parent materials vary across the region to form soil clays with high reactivity, such as vermiculite, to soil clays with low reactivity, such as kaolinite. The subtropical climate with year-round rain resulted in soil temperature and moisture that promoted microbial growth and rapid organic matter mineralization. Thus, soil organic matter levels tend to be lower compared to the northern regions of the US. Although soils are quite diverse in the region, there are many similarities in soil types across state borders that result in similar management strategies for optimizing crop productivity. Therefore, the diagnostic soil testing tools that evaluate soil fertility can often be regionally applied.

Soil testing laboratories in Alabama, Georgia, Louisiana, Mississippi, North Carolina and South Carolina use various methods of identifying soils within their states which require different calibration and interpretations. Alabama and Mississippi use an estimated soil cation exchange capacity (CEC) to group soils while Louisiana, Georgia and South Carolina use physiographic regions similar to USDA's major land resource areas. Louisiana also uses soil texture to differentiate soil type. North Carolina and Florida use a different interpretation for organic soils compared to mineral soils. Also, Florida uses a different soil test method for calcareous soils in the state.

The United States Department of Agriculture (USDA) defines Major Land Resource Areas (MLRA) that provides greater detail on the physical geography of the physiographic regions of Fig. 1. Some of the MLRAs of the Southeastern US are shown in Fig. 3. This chapter groups soils within the Coastal Plain physiographic region, the MLRAs shown in Fig. 3, and the states of Oklahoma and Texas to discuss to importance of soils in relation to crop production. In addition, the unique nature of the calcareous and organic soils within the Southeastern US is discussed.

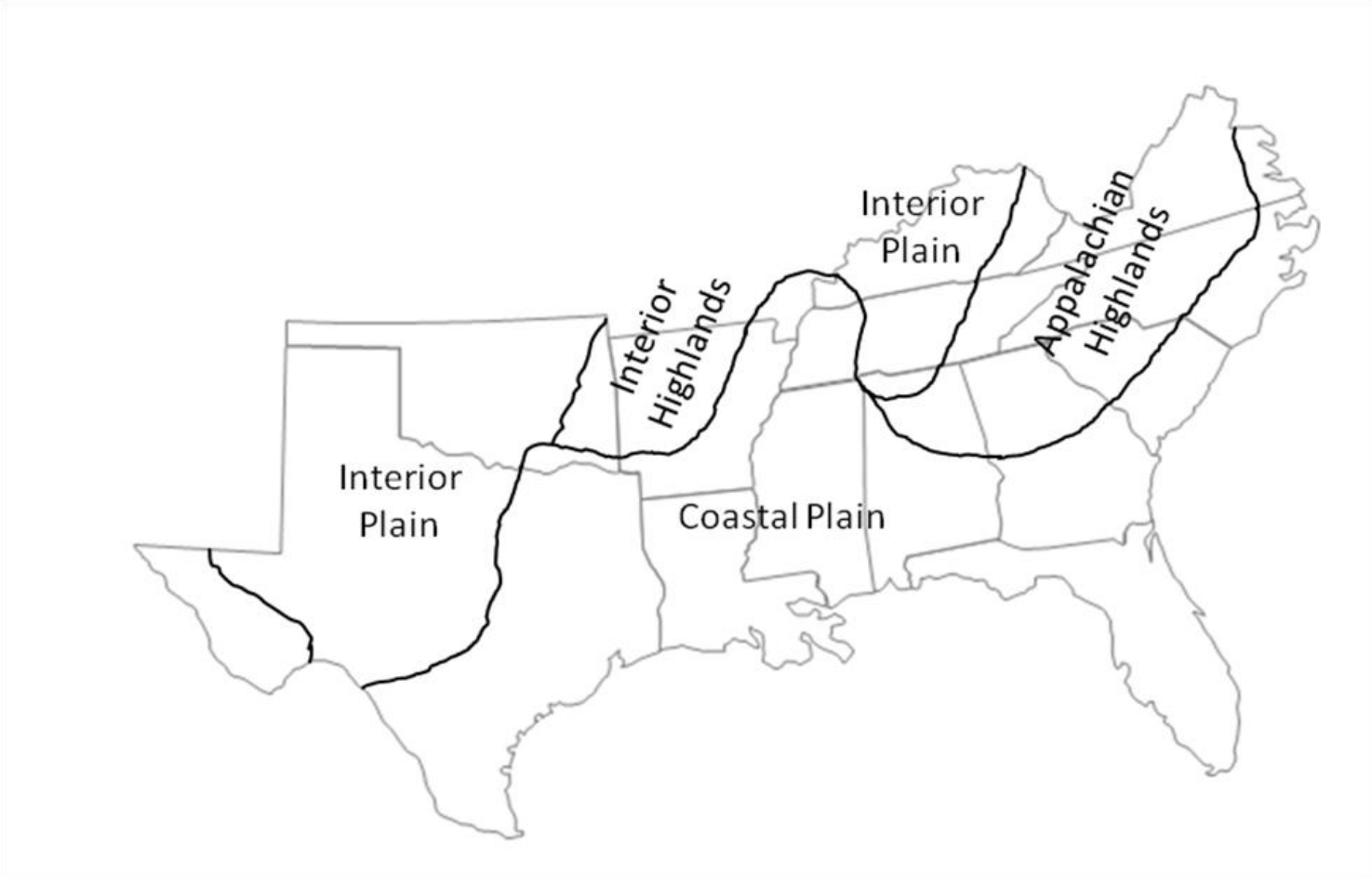



*Fig. 1. Physiographic regions of the Southeastern US (Fenneman, 1938).*

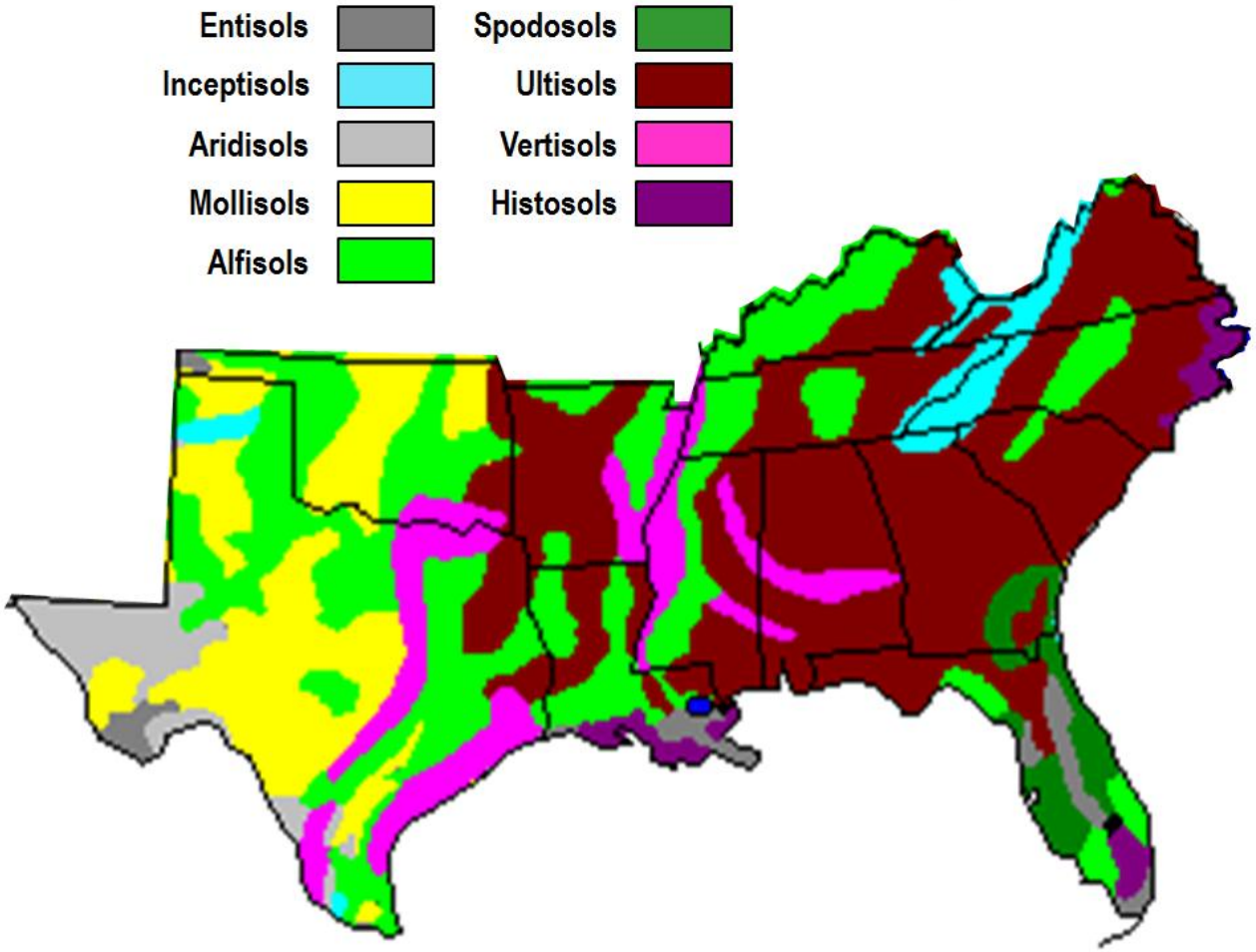
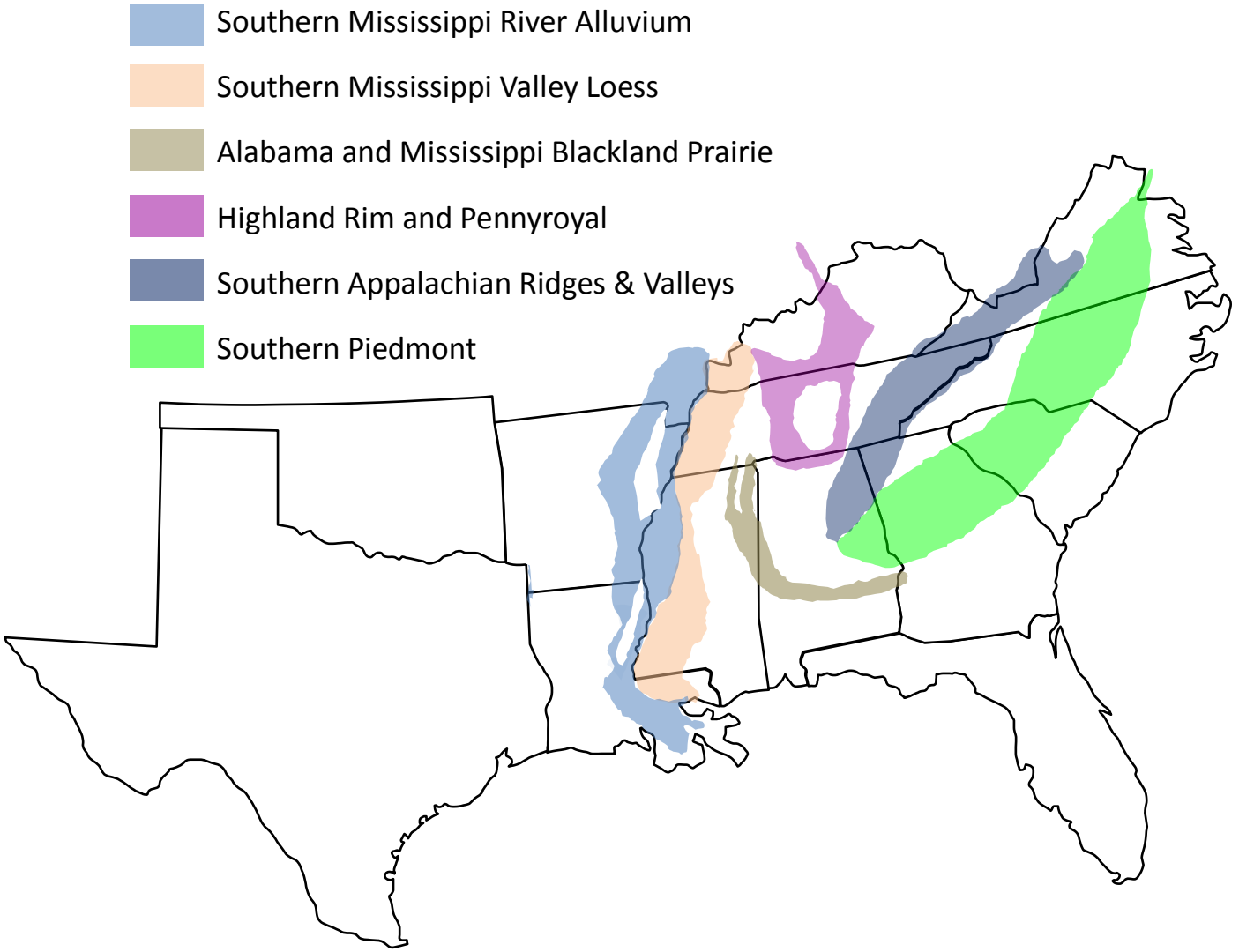



*Fig. 2. Soil Orders of the Southeastern US (modified from Dutch, 2012).*

*Fig. 3. Some Major Land Resource Areas in the Southeastern US (USDA-NRCS, 2006).*



## Coastal Plain physiographic region

The Atlantic Plain is a physiographic region that includes the Coastal Plain on land (Fig. 1) and the Continental Shelf below sea level. The Coastal Plain is the largest physiographic region in the Southeastern US and encompasses most of the crops and resultant agricultural management. The Coastal Plain region averages 155 miles in width and is as great as 400 miles wide around the Gulf of Mexico region. The Atlantic region is bound, irregularly, on the inland side with the Piedmont region at higher elevations (Daniels et al., 1973). Older rocks and sediments form the inner boundary of the Coastal Plain in the Gulf of Mexico region at lower elevations with rolling topography and swampy areas. The Gulf Coastal Plain areas are marked by larger rivers and drainage basins (West, 2000). The Coastal Plains are derived from sediments eroded from the Appalachian and Piedmont plateaus and are comprised of sand or loamy sand surface soils over 2 meters in depth that are acidic and mostly well drained.

With sandy soil texture and an acidic nature, the Coastal Plain soils require extremely good lime, fertilizer, and water management. Appropriate soil test methods have to be employed to optimize economic yields of crops produced in this region. The Mehlich-1 extractant was developed primarily for these weakly buffered soils with low CEC, but several states now use Mehlich-3 which is better on soils with higher CEC and has the ability to extract micronutrients.

## Southern Piedmont MLRA

The Southern Piedmont ranges along Virginia, the Carolinas, Georgia and Alabama and the soils are mostly well drained sandy loams with clayey or loamy subsoils that are moderately fertile (Fig. 3). The eastern boundary of the Piedmont is shared with the Coastal Plain. To the west, the Piedmont is mostly bounded by the Blue Ridge ranges of the easternmost Appalachian Mountains. The Piedmont is a part of the Appalachian Highlands physiography with the varying width being narrow above the Delaware River to nearly 300 miles wide in North Carolina. The Piedmont covers an area of approximately 80,000 square miles or nearly 52 million acres. Cecil soils form the most extensive series of the Piedmont plateau that are mapped on more than 10 million acres. About half of the acreage of Piedmont soils is cultivated, and the rest is used for pasture or forest. The most common crops are small grain, corn, cotton, and tobacco.

Like Coastal Plain soils, soils of the Piedmont are acidic with low activity clays. However, because of higher silt and clay in most surface horizons, these soils generally have a higher cation exchange capacity. Clays are often coated with oxides and hydrous oxides of iron and aluminum giving them a red or orange appearance. They have a higher P fixing capacity than Coastal Plain soils. Both the Mehlich-1 and Mehlich-3 extracts are used in states with Piedmont soils. Piedmont and Coastal Plain soils can be extracted with the same extract (Mehlich-1 or Mehlich-3) but they may require different interpretations for fertilizer recommendations.

## Southern Appalachian Ridges and Valleys MLRA

This area of the Southern Appalachian Mountains is highly diversified with many parallel ridges, narrow valleys and large areas of low, irregular hills extending from North Alabama through Tennessee, Kentucky, and Virginia (Fig. 3). The valleys were formed from limestone and cherty limestone whereas the ridges and plateaus are capped with sandstone and shale. Both the limestone valleys and the sandstone ridges and plateaus are dominated by Udult soils and to a

lesser extent, Udept soils. These are highly weathered soils of the humid region with a well-developed soil profile. Soils in the valleys tend to be deeper with more silt and clay than soils formed from sandstone and shale on the plateaus. Karst topography dominates the limestone valley. The sandier soils on the sandstone ridges and plateaus are often more acidic and infertile with a depth to the sandstone bedrock anywhere from a few inches to several feet. Both Mehlich-1 and Mehlich-3 soil test extractants have been used successfully on these soils.

## Highland Rim and Pennyroyal

The Highland Rim and Pennyroyal ranges from extreme northern Alabama, through Tennessee and Kentucky, and enters into Indiana (Fig. 3). The landscape consists of rolling hills, upland flats, and narrow valleys. The MLRA is underlain by Ordivician- to Mississippian-aged limestone. Karst areas exist where the limestone not overlain by clay can be up to 80 feet thick. Extensive cave systems exist due to the weathering of the limestone. Dominant soil orders include Inceptisols, Alfisols, and Ultisols. Soils formed from loess and limestone residiuum on hills, ridges, and plateaus or alluvium in flood plains. The soils are deep and well drained with loamy to clayey soil texture. Soils in the area are highly productive and are cropped to hay and pasture for beef cattle, corn, soybeans, wheat, and tobacco. Crider soil covers approximately 500,000 acres in 35 counties in Kentucky and is recognized as the state soil because of its exceptional fertility and productivity. Both Mehlich-1 and Mehlich-3 soil test extractants have been used successfully on these soils.

## Alabama and Mississippi Blackland Prairie MLRA

Blackland prairie extends from Tennessee through east central Mississippi into Alabama (Fig. 3). The area is commonly known as the "Black Belt" because of the dark surface colors of many of the soils. This area covers as many as 5,500 square miles. These soils were derived from alkaline or acid marine clays, which are often intermingled. These soils have calcareous subsoils with high content of montmorillonitic clays. The surface soils are acidic, somewhat poorly drained, with a high percentage of smectitic clays that shrink and crack. The area is level to undulating. The main crops are pastures, soybeans, and cotton. Mississippi and Alabama both use the Lancaster extract for P, K, and other macronutrients for these soils.

## Southern Mississippi Valley Loess MLRA

Loess soils are extensive in Tennessee and Kentucky making up about 500,000 acres and occurring in 35 counties of the states (Fig. 3). The soils are also present in Mississippi and Louisiana. These soils are very productive if they are not eroded. These brown silt loam soils are used to produce corn, small grain, soybeans, tobacco, and hay. The Crider soil series exists in this MLRA and consists of deep, well drained, moderately permeable soils, with 0-20% slopes, formed in a mantle of loess overlying a limestone base. Other important series include Memphis, Loring, Grenada, Calloway, and Henry drainage catena. These, and similar series of Alfisols are common in all of the four states mentioned above. The loess in these areas is deposited over coastal plain deposits. The loess thickness decreases from west to east. For example, in Tennessee the loess is about 100 feet thick in the loess bluffs next to the Mississippi river bottoms and progressively thins to 1 foot or less moving east to the Tennessee River. These

soils have a wide abundance of naturally occurring hard pans, called fragipans, which restrict root growth. When the fragipans are near the soil surface due to erosion, they can severely limit the effective rooting zone and reduce crop productivity. Both Mehlich-1 and Mehlich-3 soil test extractants have been used successfully on these soils.

## Southern Mississippi River Alluvium MLRA

Alluvial soils formed along the Mississippi delta and spread across 26,000 square miles in Louisiana, Arkansas, Mississippi, Tennessee, and Kentucky (Fig. 3). The dominant soil orders in this MLRA are Vertisols, Alfisols, Inceptisols, and Entisols. The soils are mostly level, made up of sandy and clayey fluvial sediments that can be several feet thick. Cotton, soybeans, corn, and rice are the main crops grown on these soils. Rice is grown in flooded areas. Careful management of nutrients, crop residues, and tillage is essential. Both Mehlich-1 and Mehlich-3 soil test extractants have been used successfully on these soils.

## Soils of Oklahoma and Texas

Oklahoma has an extremely variable climate and many different kinds of geologic materials across the state. Annual precipitation ranges from 12 inches in the northwest to 65 inches in the southeast. Different soil forming factors greatly influenced the kinds of soils found in the state. Seven of the 12 soil orders are represented in Oklahoma. There are over 2,500 different types of soil in the state. Some soils are naturally fertile, but others have one or more limiting factors in productivity. A vast majority of soils are under forest and pasture systems. Soil types and their properties in Oklahoma can be grouped by major land resource areas and geographical regions. Soils were developed under short and medium height grasses with low rainfall in western Oklahoma. Therefore, soils in this region tend to have high pH and caliche layers at various depths. These soils are typically high in exchangeable K which is probably due to low precipitation and the parent materials. Mehlich-3 extractable soil test K value can be as high as 600 mg $kg^{-1}$, so farmers seldom use K fertilizers. In central Oklahoma, soils were formed under tall grasses and variable parent materials. These soils are very productive occupying the main winter wheat producing area of the state. Due to continuous wheat production and anhydrous ammonia use, many of these soils have become very acidic. Low soil pH and aluminum toxicity have become yield limiting factors. Liming is encouraged in order to sustain production. Coastal plain soils, and soils developed on the Ozark Highlands can be found in eastern Oklahoma. Most soils in this region are rocky and shallow in depth. Collecting a representative soil sample is a challenge. Phosphorus and K fertility levels in east Oklahoma are generally low. Port Silt Loam (Fine-silty, mixed, superactive, thermic Cumulic Haplustolls) is Oklahoma's state soil, which covers over one million acres and appears in 33 of the 77 counties. Port Silt Loam is deep, well drained, highly productive, and suited for a wide range of crops. Oklahoma uses the Mehlich-3 method for testing soil fertility.

Texas soils are extremely diverse due to climate and parent material. Annual precipitation ranges from 56 inches in southeast Texas near Beaumont to less than 8 inches in far west Texas near El Paso. Frost free day ranges from over 320 near Brownsville to less than 185 days in the Panhandle. Most Texas soils are classified into 7 major soil orders (Alfisols, Aridisols, Entisols, Inceptisols, Mollisols, Ultisols, and Vertisols), but small areas of 2 other orders (Histosols and Spodosols) are also present. There are 15 land resource areas in the state, and approximately

1,300 mapped soil series. The proposed Texas state soil is the Houston Black, a classic Vertisol covering 2 million acres in central Texas from Bonham and Dallas to San Antonio. Much of the state is in native rangeland (65%) with only about 25% of agricultural lands used for crop production and pastures. Approximately 40% of the 168 million acres of soils in Texas are calcareous. Although soils are acidic in the eastern part, most Texas soils have neutral or alkaline pH. Most of the native soils have low P availability unless heavily fertilized. Potassium availability is high in most of the native soils with the exception of those in the eastern portion. Finding a soil test extractant that works on this diversity of soils has been a challenge. Texas used a modified Morgan extractant (1.4 M $NH_4OAc$ + 1 M HCl + 0.025 M $H_4EDTA$, pH=4.2, 1 hr. shaking) for many years but converted to the Mehlich-3 extractant in 2004.

## Organic Soils

The Everglades Agricultural Area in Florida spreads across 280,000 acres of land south and east of Lake Okeechobee in south Florida. This region is comprised of Histosols (Fig. 2) which are organic soils with organic matter contents ranging from 30 to 90% having shallow water tables. Sugarcane and high value vegetable crops are produced on these soils under intensive management practices. Due to its proximity to the Everglades ecosystem, soil testing is promoted to limit any excess nutrient applications to the agricultural fields. Areas of eastern North Carolina also have a high abundance of Histosols (Fig. 2) and are heavily cultivated. The organic soils in Florida are extracted using water for P and acetic acid for K, Mg, Si and Na. Organic soils in North Carolina are extracted with Mehlich-3.

## Calcareous soils

Calcareous soils are associated with the Blackland Prairie soils of Mississippi and Alabama (Black Belt region) and the Texas Black soils. Other areas of Texas and Oklahoma have calcareous soils. The marl and rocky calcareous soils in Miami-Dade County in Florida usually contain from 30% to 94% $CaCO_3$. The pH values of calcareous soils are greater than 7 and usually in the range from 7.4 to 8.4. Textures of calcareous soils can be sandy, loamy or gravelly in South Florida to clayey in the Texas Black soils and Blackland Prairie soils of Mississippi and Alabama. Soil depths range from less than five inches to several feet. The South Florida soils are important for production of vegetables, fruits, and ornamentals. Over 85% of Florida's tropical fruits are grown on calcareous soils in the southern part of the state. This crop is grown in the area because of favorable temperatures, rather than favorable soil characteristics. Careful management of nutrients is critically important to the successful production of crops on calcareous soils. In Florida, ammonium bicarbonate-DTPA solution is used as a soil extractant; however only P is interpreted since no calibrations exist for the other nutrients.
In the Western US, where calcareous and alkaline soils dominate, Olsen extraction is the preferred procedure for P while a separate extraction with 1 M ammonium acetate is used for cation extraction. Calcareous soils in Texas and Oklahoma are extracted with Mehlich-3. Alabama and Mississippi use the Lancaster extract for the calcareous soils in the Black Belt region. Mehlich-1 does not perform well in extracting available P from calcareous soils.

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*Chapter 1.2*

# Soil Test Correlation and Calibration for Recommendations

*C.C. Mitchell and R. Mylavarapu*

Three terms often used in soil testing are "correlation", "calibration", and "interpretation" (Brown, 1987). These terms describe very important concepts that form the basis of soil testing programs. The Soil Science Society of America (SSSA, 1997) provides the definitions for the terms as shown below.

*Soil test correlation: The process of determining the relationship between plant nutrient uptake or yield and the amount of nutrient extracted by a particular soil test method.*

*Soil test calibration: The process of determining the crop nutrient requirement at different soil test values.*

*Soil test interpretation: The process of developing nutrient application recommendations from soil test concentrations, and other soil, crop, economic, environmental and climatic information.*

Putting soil test values to practical use for growers involves all three processes. Correlation provides evidence that a particular soil test method is useful in predicting nutrient availability and thus crop growth. For a soil test method to have value, a good relationship must exist between plant response and soil test values. Calibration provides information on how much nutrient should be applied at a particular soil test value to optimize crop growth. This information is gathered by monitoring crop yield with addition of increasing quantities of a nutrient to soil at a specific soil test range. The resulting data is plotted with yield versus nutrient application rates and is referred to as a yield response curve.

Soil test interpretation involves the use of information from correlation and calibration to make recommendations for nutrient applications. Because of a multitude of factors to consider for soil test interpretation, there are often different beliefs or philosophies on how to achieve the best recommendation. Most Universities interpret soil test calibration data just described to determine the application rate of a nutrient to achieve a yield that is approximately 95% of maximum yield. This nutrient application rate is the economic optimum rate where the grower would achieve the greatest economic return from the crop versus the cost of the nutrient. This philosophy is referred to as the sufficiency approach because just enough nutrients are recommended that would be sufficient for economically optimum crop growth. Other philosophies for nutrient recommendations focus on soil test values. Examples include maintaining soil test values with application of nutrients removed in the crop, building up soil test values with heavy nutrient applications, or maintaining certain cation ratios. University recommendations often include some combination of the sufficiency approach with soil test maintenance or build up but greater emphasis is placed on the sufficiency philosophy. Soil test correlation, calibration, and interpretation have been described in detail in previous publications (Corey, 1987; Cope and Rouse, 1973; Eckert, 1987; Evans, 1987; Kopittke and Menzies, 2007; Olson et al., 1987).

Most soil test correlation and calibration data in the southern US were collected in the 1950s, 60s, and 70s by researchers working at state agricultural experiment stations. Some states continually update and revisit their calibration through research verification trials, new soil fertility research, or long-term research. However, public support for this type of research is not readily available. Results from earlier research were often published as state experiment station or extension circulars and may be difficult to access outside of the state where it was published. Also, data were often extracted from other soil fertility research projects and may not appear simply as "soil test calibration" research. An example is J.T. Cope's Alabama Agricultural Experiment Station Bulletin 561 (1984) which summarized several long-term soil fertility experiments. These experiments also provided important data for soil test calibration in Alabama but soil testing is not mentioned in the title.

The soils, crops, and climates in the southern region are diverse. Because of this diversity and because of the autonomy of state agricultural experiment stations and state Land Grant Universities, soil test interpretations are largely developed independently within each state. Most Universities summarize their correlation and calibration research with extension publications or web sites that provide recommendations from soil test results. Most state laboratories provide this information on their web site which can be accessed via the SERA-IEG-6 website for laboratories of the Southeastern US (SERA-IEG-6, 2013). Coastal Plain soils are similar in soil fertility but span across several state borders in the Southeastern US. Data from several states has been compiled on soil test correlation, calibration, and interpretation for peanuts and cotton on these soils (Mitchell, 1994; Mitchell, 2010).

Soil test fertility indices for P and K used by public soil test laboratories in the Southeastern US states are presented in Table 1 for the major agronomic crops grown in each state (Chapter 1.2). The indices include ratings of very low (VL), low (L), medium (M), high (H), and very high (VH). These ratings define whether nutrient levels are deficient or sufficient for optimal plant growth. The agronomic critical value occurs at the boundary between medium and high. With the sufficiency approach, fertilizer nutrients are usually recommended below this level and not recommended above this level. More fertilizer nutrients are recommended with a low versus medium rating. The amount of fertilizer nutrient recommended is determined or validated with calibration field plots with specific crops. The soil fertility indices are periodically updated and presented in Savoy (2013) on the SERA-IEG-6 website (SERA-IEG-6, 2013).

*Table 1. Soil test fertility indices for Phosphorus (P) and Potassium (K) with various methods used in 13 states of the Southeastern US and Puerto Rico (adapted from Savoy, 2013).*

| State | Method | Soil | CEC | Crop | P, mg $kg^{-1}$ | | | | | K, mg $kg^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $cmol_c\ kg^{-1}$ | | VL | L | M | H | VH | VL | L | M | H | VH |
| **AL** | Mehlich-1 | All except Black Belt clays | ≤ 9 | Peanuts and pine trees | < 3 | 3-5 | 6-9 | 10-25 | > 25 | | | | | |
| | " | " | > 9 | Peanuts and pine trees | < 1 | 1-2 | 3-5 | 6-15 | > 15 | < 20 | 21-28 | 29-40 | 41-100 | > 100 |
| | " | " | ≤ 9 | All except peanuts and pine trees | < 7 | 7-12 | 13-25 | 26-50 | > 50 | | | | | |
| | " | " | > 9 | All except peanuts and pine trees | < 4 | 4-7 | 8-15 | 16-30 | > 30 | | | | | |
| | " | " | < 4.7 | Cotton, legumes, and vegetables | | | | | | < 15 | 16-30 | 31-60 | 61-120 | > 120 |
| | " | " | " | Grasses, corn, and soybean | | | | | | < 10 | 11-20 | 21-40 | 41-80 | > 80 |
| | " | " | " | Peanuts and pine trees | | | | | | < 10 | 11-14 | 15-20 | 21-50 | > 50 |
| | " | " | 4.7-9 | Cotton, legumes, and vegetables | | | | | | < 22 | 23-45 | 46-90 | 91-180 | > 180 |
| | " | " | " | Grasses, corn, and soybean | | | | | | < 15 | 16-30 | 31-60 | 61-120 | > 120 |
| | " | " | " | Peanuts and pine trees | | | | | | < 15 | 16-21 | 22-30 | 31-75 | > 75 |
| | " | " | > 9 | Cotton, legumes, and vegetables | | | | | | < 30 | 31-60 | 61-120 | 121-240 | > 240 |
| | " | " | " | Grasses, corn, and soybean | | | | | | < 20 | 21-40 | 41-80 | 81-160 | > 160 |
| | Lancaster | Black Belt Clays | All | All | < 10 | 10-18 | 19-36 | 37-72 | > 72 | | | | | |
| | " | " | " | Cotton, legumes, and vegetables | | | | | | < 40 | 41-80 | 81-120 | 121-240 | > 240 |

*Table 1 continued*

| State | Method | Soil | CEC | Crop | P, mg $kg^{-1}$ | | | | | K, mg $kg^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $cmol_c\ kg^{-1}$ | | VL | L | M | H | VH | VL | L | M | H | VH |
| | " | " | " | Grasses, corn, and soybean | | | | | | < 26 | 26-60 | 61-95 | 96-160 | > 160 |
| | " | " | " | Peanuts and pine trees | | | | | | < 26 | 26-37 | 38-60 | 61-110 | > 110 |
| **AR** | Mehlich-3 | All | All | Cotton, corn, soybean, wheat, rice, grain sorghum, forages, and turf grasses | < 16 | 16-25 | 26-35 | 36-50 | > 50 | < 60 | 61-90 | 91-130 | 131-175 | > 175 |
| | " | " | " | Turf grasses | < 16 | 16-25 | 26-35 | 36-50 | > 50 | < 21 | 21-40 | 41-60 | 61-100 | > 100 |
| | " | " | " | Commercial vegetables | < 20 | 20-30 | 31-40 | 41-75 | > 75 | < 61 | 61-90 | 91-130 | 131-175 | > 175 |
| **FL** | Mehlich-3 | All | All | All | | < 26 | 26-40 | >40 | | | <26 | 26-40 | >40 | |
| **GA** | Mehlich-1 | Coastal Plain | All | All except peanut, cotton, legumes, stone fruits, nuts, and vegetables | | < 16 | 16-30 | 31-50 | > 50 | | < 31 | 31-75 | 76-125 | > 125 |
| | " | " | " | Cotton, legumes, stone fruits, nuts, and vegetables | | < 16 | 16-30 | 31-50 | > 50 | | < 36 | 36-85 | 86-137 | > 137 |
| | " | " | " | Peanut | | < 8 | 8-15 | 16-30 | > 30 | | < 16 | 16-37 | 38-62 | > 62 |
| | " | Piedmont | " | All except peanut, cotton, legumes, stone fruits, nuts, and vegetables | | < 11 | 11-20 | 21-37 | > 37 | | < 51 | 51-100 | 101-175 | > 175 |
| | " | " | " | Cotton, legumes, stone fruits, nuts, vegetables | | < 11 | 11-20 | 21-37 | > 37 | | < 61 | 61-125 | 126-200 | > 200 |
| | " | " | " | Peanut | | < 6 | 6-10 | 11-17 | > 17 | | < 26 | 26-50 | 51-87 | > 87 |

*Table 1 continued*

| State | Method | Soil | CEC | Crop | P, mg kg$^{-1}$ | | | | | K, mg kg$^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | cmol$_c$ kg$^{-1}$ | | VL | L | M | H | VH | VL | L | M | H | VH |
| **KY** | Mehlich-3 | All | All | Corn, soybean | < 3 | 3-13 | 14-30 | > 30 | | < 50 | 50-95 | 96-150 | > 150 | |
| | " | " | " | Burley tobacco | < 4 | 4-14 | 15-28 | 28-40 | > 40 | < 48 | 48-102 | 103-151 | 152-225 | > 225 |
| | " | " | " | Alfalfa | < 5 | 5-13 | 14-30 | > 30 | | < 49 | 49-101 | 102-148 | 149-223 | > 223 |
| **LA** | Mehlich-3 | Coastal Plain | 4 † | All | < 6 | 6-20 | 21-40 | > 40 | | < 46 | 46-68 | 69-113 | > 113 | |
| | " | Flatwoods | 6 | " | < 6 | 6-17 | 18-35 | > 35 | | < 57 | 57-91 | 92-136 | > 136 | |
| | " | Miss. Terraces | 8 | " | < 6 | 6-17 | 18-35 | > 35 | | < 69 | 69-113 | 114-159 | > 159 | |
| | " | " | 10 | " | " | " | " | " | | < 92 | 92-136 | 137-182 | > 182 | |
| | " | Coastal prairies | 8 | " | < 6 | 6-15 | 16-35 | > 35 | | < 69 | 69-113 | 114-159 | > 159 | |
| | " | " | 10 | " | " | " | " | " | | < 92 | 92-136 | 137-182 | > 182 | |
| | " | " | 15 | " | " | " | " | " | | < 114 | 114-182 | 183-227 | > 227 | |
| | " | Alluvial | 4 | " | < 21 | 21-30 | 31-60 | > 60 | | < 46 | 46-68 | 69-113 | > 113 | |
| | " | " | 8 | " | " | " | " | " | | < 69 | 69-113 | 114-159 | > 159 | |
| | " | " | 10 | " | " | " | " | " | | < 92 | 92-136 | 137-182 | > 182 | |
| | " | " | 15 | " | " | " | " | " | | < 114 | 114-182 | 183-250 | > 250 | |
| | " | " | 20 | " | " | " | " | " | | < 160 | 160-227 | 228-341 | > 341 | |

*Table 1 continued*

| State | Method | Soil | CEC | Crop | P, mg $kg^{-1}$ | | | | | K, mg $kg^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $cmol_c\ kg^{-1}$ | | VL | L | M | H | VH | VL | L | M | H | VH |
| **MS** | Lancaster | All | All | All except rice | <10 | 10-18 | 19-36 | 37-72 | > 72 | | | | | |
| | " | " | " | Rice | <5 | 5-9 | 10-18 | 19-22 | > 22 | | | | | |
| | " | " | < 7 | Group A ‡: Perennial winter and summer grasses, annual legumes with ryegrass, peanuts, rice | | | | | | < 21 | 21-40 | 41-60 | 61-105 | > 105 |
| | " | " | 7-14 | " | | | | | | < 26 | 26-55 | 56-80 | 81-140 | > 140 |
| | " | " | 15-25 | " | | | | | | < 31 | 31-65 | 66-90 | 91-157 | > 157 |
| | " | " | > 25 | " | | | | | | < 36 | 36-75 | 76-100 | 101-175 | > 175 |
| | " | " | < 8 | Group B ‡: Corn, soybeans, forages not in Groups A or C | | | | | | < 26 | 26-55 | 56-80 | 81-140 | > 140 |
| | " | " | 8-14 | " | | | | | | < 31 | 31-70 | 71-95 | 96-167 | > 167 |
| | " | " | 15-25 | " | | | | | | < 36 | 36-80 | 81-105 | 106-185 | > 185 |
| | " | " | > 25 | " | | | | | | < 41 | 41-90 | 91-120 | 121-210 | > 210 |
| | " | " | < 8 | Group C ‡: Alfalfa, cotton, hybrid bermudagrass hay | | | | | | < 36 | 36-75 | 76-100 | 101-175 | > 175 |
| | " | " | 8-14 | " | | | | | | < 46 | 46-95 | 96-120 | 121-210 | > 210 |
| | " | " | 15-25 | " | | | | | | < 61 | 61-120 | 121-145 | 146-255 | > 255 |
| | " | " | > 25 | " | | | | | | < 76 | 76-130 | 131-160 | 161-280 | > 280 |

*Table 1 continued*

| State | Method | Soil | CEC | Crop | P, mg $kg^{-1}$ | | | | | K, mg $kg^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $cmol_c\ kg^{-1}$ | | VL | L | M | H | VH | VL | L | M | H | VH |
| **NC** | Mehlich-3 | All | All | All | < 11 | 11-27 | 28-53 | 54-107 | > 107 | < 18 | 18-43 | 44-87 | 88-174 | > 175 |
| **OK** | Mehlich-3 | All | All | All | < 11 | 11-20 | 21-32 | > 32 | | < 26 | 26-75 | 76-125 | 126-175 | > 176 |
| **PR** | Bray P1 | All | All | All | | < 6 | 6-10 | > 10 | | | < 77 | 78-156 | > 156 | |
| | Bray P2 | " | " | " | | < 11 | 11-20 | > 20 | | | < 77 | 78-156 | > 156 | |
| | Olsen | " | " | " | | < 7 | 7-17 | > 17 | | | < 77 | 78-156 | > 156 | |
| **SC** | Mehlich-1 | Coastal Plain | All | All except peanut | | < 16 | 16-30 | 31-60 | > 60 | | < 36 | 36-78 | 79-117 | > 117 |
| | " | Piedmont | " | All except peanut | | < 11 | 11-20 | 21-40 | > 40 | | < 36 | 36-78 | 79-117 | > 117 |
| | " | All | " | Peanut | | < 6 | 6-9 | 10-24 | > 24 | | < 15 | 15-20 | 21-50 | > 50 |
| **TN** | Mehlich-1 | All | All | All except cotton | | < 10 | 10-15 | 16-60 | > 60 | | < 46 | 46-80 | 81-160 | > 160 |
| | " | " | " | cotton | | " | " | " | " | | < 71 | 71-90 | 91-159 | > 159 |
| | Mehlich-3 | " | " | All except cotton | | < 20 | 20-30 | 31-105 | > 106 | | < 58 | 58-101 | 102-202 | > 202 |
| | " | " | " | cotton | | " | " | " | " | | < 90 | 90-178 | 179-202 | > 202 |
| **VA** | Mehlich-1 | All | All | All | < 2 | 2-5 | 6-17 | 18-55 | > 55 | < 8 | 8-37 | 38-87 | 88-155 | > 155 |

† CEC of 15 and 20 meq (100 g)$^{-1}$ for Alluvial correspond to silt loams and clays, respectively. CEC of 15 meq (100 g)$^{-1}$ for Coastal prairies corresponds to clay loams. CEC of 10 meq (100 g)$^{-1}$ correspond to silt loams. CEC of 8 meq (100 g)$^{-1}$ for Miss. Terraces corresponds to silt loams. CEC of 6 meq (100 g)$^{-1}$ for Flatwoods and 8 meq (100 g)$^{-1}$ for Coastal prairies and Alluvial corresponds to very fine sandy loams. CEC of 4 meq (100 g)$^{-1}$ corresponds to sandy loams.
‡ A more complete list of crop groupings can be found in Chapter 4.4.

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# *Unit 2*

# Laboratory Operation

*Chapter 2.1*

# Soil Preparation, Measurement, and Storage

*R. Mylavarapu and R. Miller*

A preliminary step in chemical analysis of any material is to prepare a homogenous sample to reduce variability in results. Since soil is a complex matrix, soil samples have to undergo a preliminary step of homogenization before analysis. Soil test laboratories homogenize soil samples by first drying the soil and then pulverizing the soil to a consistently small particle size.

Soil samples are dried by placing them into hot air ovens at $40^{o}$ C ($104^{o}$F) or less. Soils dried in this manner are referred to as air-dried soils. Soil can be transferred to boxes or trays to be placed into the oven. The entire bag or box containing the sample can also be placed in the oven with the top left open to allow moisture to be released. Typically, 300 to 500 g of each soil sample is placed in the oven for drying. Drying ovens are typically equipped with exhaust fans to expedite air moisture removal. Three factors that control drying rate are exposed surface area of the sample, air flow rate, and relative humidity of the air. Drying temperatures are maintained at $40^{o}$ C or less because any higher temperature may significantly increase or decrease extractable potassium (K) (Bates, 1961; Steenkamp, 1927) in certain soils dependent on their mineralogy. Illitic and vermiculitic clay minerals are known for either release or fixation of K when soils are heated for drying. The method of drying soils may also affect results for extractable phosphorus (Searle and Sparling, 1987), extractable zinc (Gogan, 1975), and mineralizable nitrogen (Kenney and Bremner, 1966). A summary of drying effects on various soil test results is presented by Brown (2001). Using soil under field moist conditions may be optimal for some analyses (Bartlett and James, 1980). Determining K in field moist soils gave better correlation to plant-available K than using air-dried soils (Luebs et al., 1956). Testing K on field moist soil was the method used in Iowa up to 1990 (Gelderman and Mallarino, 1998). Although testing field moist soil may be optimal for some analyses, the method of preparing a large number of field moist soils is cumbersome and time-consuming. Thus, drying soil in a hot air oven prior to homogenizing and testing is preferred.

After drying, soil samples are reduced to a small particle size through pulverization or grinding. Before this step, it is advisable to remove plant and root material, stubbles, rocks, stones and any other extraneous material in the samples. Reducing the sample to a small particle size may be accomplished by use of power-driven mortar-pestle, rotating flail-type pulverizer, plate grinder, or manual use of a stone rolling pin. After pulverization or grinding, the sample is passed through a 2 mm sieve (US No. 10 mesh) which is the standard particle size for most soil testing methods. Care must be taken to not contaminate the sample by grinding or screen surfaces that come into contact with the sample. In particular, galvanized metal containing zinc and brass can contribute copper and zinc to the sample. Contamination from one sample to the next may also be a concern if the pulverization equipment is not properly evacuated before moving to the next sample.

Variability of soil test results increases as sample size decreases (Miller et al., 2012). The minimum milligrams of sample used for an analysis should be at least 1000 times the millimeter sieve opening through which the sample was passed (Jackson, 1958). For soil ground to pass a 2 mm screen, the minimum sample size should be 2 g. Soil test methods for extractable plant

nutrients use at least 2 g of soil. Methods using sample sizes less than 2 g require soil to be ground to a smaller particle size. An example is carbon and nitrogen analysis on combustion instruments.

Analytical methods are most precise when the sample being analyzed is weighed on an analytical balance. Measuring soil by weight is too time-consuming when hundreds of samples are analyzed per day. Therefore, a soil sample for analysis is commonly measured with a scoop. The soil is stirred to ensure mixing and a scoop removes a portion of the soil. The scoop is tapped three times to settle soil into the scoop and excess soil is leveled off the top of the scoop. The scoop can measure a sample on a volume or weight basis. Volume measured basis means that the amount of soil measured is simply the volume of the scoop. Weight measured basis means a soil density is assumed and the volume of the scoop corresponds to a certain mass of soil. For example, if a soil density of 1.18 g/cm$^3$ is assumed, a soil scoop of 1.7 cm$^3$ would measure 2 g of soil. Most soil test laboratories report concentrations of analytes in soil on a weight basis of soil. A typical example of this is the unit of mg of nutrient per kg of soil (mg/kg = ppm). When presenting analyte concentrations on a weight basis using a scoop for soil measurement, an assumption is required on the density of soil measured in the scoop. North Carolina does not report concentrations on a soil weight basis because soil density can vary considerably with the abundance of both mineral and organic soils in agricultural production. Therefore, analyte concentrations in soil are determined on a volume basis of soil measured with units of mg nutrient per cubic decimeter of soil (mg/dm$^3$). Mehlich developed his soil test extractants in North Carolina and was a strong proponent of utilizing volume as a basis for soil test measurements (Mehlich, 1973; Mehlich, 1980).

It is often preferable to keep samples stored in case retesting needs to be performed. There should be no change in soil test results in air-dried soils with short-term storage up to 6 months. However, effects of longer term storage have been shown to be variable. Soil pH increased after 7 years of storage (Slattery and Burnett, 1992) and decreased after 20 years of storage (Prodromou and Pavlatou-Ve, 1998) with changes ranging from 0.2 to 0.6 pH units. Laboratories in Canada report no change in soil test values with storage over 9 years (Bates, 1993). Brown (2001) presents a summary of results on how storage may affect soil test values. It is advisable to store air-dried soils under a condition that minimizes microbial activity such as sealed containers that avoids moisture absorbed by the soil. Freezing soil may not be appropriate for minimizing microbial activity in long term storage since Allen and Grimshaw (1962) observed release of ammonium and phosphorus in frozen soil samples.

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*Chapter 2.2*

# Quality Assurance and Quality Control in a Soil Test Laboratory

*D.H. Hardy, R. Mylavarapu, and T. Provin*

## Introduction

Soil testing laboratories provide soil fertility recommendations for farmers and consultants to make management decisions in crop production. Increasingly, these same laboratories are providing results relevant to environmental issues such as phosphorus loss from agricultural soils and heavy metal loading from waste application to soils, as governed by state and federal regulations. Although end uses of soil test results may vary, the primary goal of the laboratory is to provide reliable, consistent, and valid data.

Quality assurance (QA) in a laboratory is governed at the management level to ensure data provided to the customer meets a defined level of confidence. Several activities of quality control (QC) are conducted in the laboratory to meet the QA objective of delivering quality data to the client. Some of these activities include analysis by trained staff, use of standard operating procedures (SOPs), use of good laboratory practices (GLPs), and statistical analysis of accuracy and precision on reference samples.

Meeting the goal of providing quality data to the client requires a well-documented and executed QA/QC plan. This written document is useful for communicating how the laboratory conducts its business of producing quality data to laboratory staff, upper levels of management, clients, and granting agencies. The North American Proficiency Testing (NAPT) program provides a good outline of a model QA/QC plan that can be used for soil testing laboratories (Watson et al., 2005).

In this chapter, we provide information on key components of QA and QC in the operation of soil testing laboratories analyzing routine grower samples for agronomic recommendations. Quality control activities involved in sample preparation and laboratory operation are described first followed by assessment of data quality.

## Quality Control in the Laboratory

Consistency in daily work, regardless of task, is extremely important to attain precise data. Quality control activities minimize errors and foster reproducibility in all aspects of laboratory work. The following practices and guidelines are important for attaining good QC.

### Standard Operating Procedures

Standard operating procedures (SOPs) are written documents that provide the foundation of QC for both technical and nontechnical work. The word "standard" implies an operation is performed the same way on each occasion. Standard operating procedures are needed for sample receiving and drying, sample grinding, sample scooping, solution preparation, soil acidity measurements, soil extraction, element analysis by inductively couple plasma-atomic emission spectrometry (ICP-AES), soil disposal, and dishwashing.

An SOP needs to describe information in sufficient detail so an employee unfamiliar with the method or task could obtain acceptable results and conduct a reliable review of results using the procedure. An SOP outlines steps to be taken to perform a task in detail to minimize individual preferences or judgments being imposed in the operation of the task. The US Environmental Protection Agency (US EPA) provides guidance on preparing SOPs (US EPA, 2007). The SOP for a given operation should include the following.

1. Title page with method, lab name, authorization signature, and approval dates
2. Table of contents
3. Scope of application
4. Summary of method
5. Safety/waste handling
6. Interferences, apparatus/equipment, reagents/chemicals
7. Sample collection, preservation, shipment, and storage
8. Calibration
9. Sample preparation
10. Procedure
11. Calculations
12. Quality control
13. Data validation
14. Preventative maintenance of instruments

The method chapters in this manual can be used as initial drafts to develop SOPs for individual laboratory environments.

Instruments and Equipment

Optimizing instrumentation or equipment is essential for producing consistent, accurate data. Users should refer to the operation manual for the manufacturer's guidelines in correct operation, calibration, maintenance, and trouble shooting. Many manufacturers offer on-site training with the purchase of an instrument as well as through regional seminars. Laboratories should take advantage of such training opportunities.

Table 1 lists guidelines for daily operation of laboratory equipment in a high-volume laboratory setting. A more stringent calibration schedule may be desirable for laboratories analyzing research samples or a small sample volume.

Documentation associated with laboratory instrumentation is extremely important. A record of purchase, repair, maintenance and calibration should be archived for all equipment and instruments. Activities that occur infrequently, such as purchase and repair, can be recorded in the manufacturer's operator manual. A notebook can serve as an instrument log for recording more frequent activities such as maintenance and calibration. Date, initials of operator, and details are needed for each activity entered in the instrument log.

As equipment ages, repairs often become more frequent. Management needs to be informed of increasing downtime and potential need for replacement. Loss of sensitivity and linearity in response may also occur as instrumentation ages.

*Table 1. Guidelines for equipment calibration and maintenance.*

| Equipment | Calibration | Special Concerns | Maintenance |
|---|---|---|---|
| ICP-AES | Calibrate after initial warm-up.<br>Check calibration every 30 samples.<br>A recalibration is necessary if periodic QC samples fail to be within control limits.<br>Calibrate after significant changes in torch, tubing, gas flow, or method. | Check temperature and humidity.<br>All unknowns since last acceptable QC sample must be reanalyzed. | Schedule routine professional maintenance yearly or as suggested by manufacturer. |
| Spectrophotometers | Calibrate after initial warm-up.<br>Check calibration every 30 samples. | | |
| pH Meter & probes | Calibrate hourly or every 60-90 samples.<br>Use pH buffer of 4.0 and 7.0; consider pH buffer 10.0 for soils with high pH. | Check temperature.<br>Check slope of calibration.<br>Electrode response varies in dilute solutions versus relatively concentrated pH buffers. | Check probes frequently for wear.<br>Make sure electrode's internal filling solution is replaced regularly, of proper formulation, and has proper height in electrode. |
| Conductivity meter | Check known samples for calibration frequently. | Check temperature and fouling of probe surfaces. | Check frequently for wear and tear of the probes. |

*Table 1 (continued). Guidelines for equipment calibration and maintenance.*

| Equipment | Calibration | Special Concerns | Maintenance |
|---|---|---|---|
| Balances | Calibrate daily prior to use. | | Clean after each use.<br>Ensure annual certification and cleaning by qualified company. |
| Solution dispensers | Calibrate daily using balance and deionized water or solution of known density. | | |
| Automated pipettes | Micropipettes should be calibrated quarterly. | Avoid handling pipettes where solution can get into the body of the pipette. | |
| Soil scoops | Calibrate annually. | Check wear on top of the scoop. | |

Chemicals, Standards, and Lab Solutions

Analytical methods should specify the quality or grade of reagents needed to achieve desired results. Instruments that rely on gases for their operation will specify the purity of gas needed in the operator's manual. Consult the manufacturer if this information is not found.

A record of chemical purchases is needed. Chemical inventory records should be kept so lab supervisors are aware of supplies on hand. Ordering should be adequate to prevent uninterrupted laboratory operation. An inventory of chemicals for the entire laboratory should occur yearly, preferably after the busiest time of year. Chemicals no longer needed, due to changes in methods or expiration dates of the reagents, should be disposed of.

Upon receipt, each chemical container should be inspected for its contents. On the container, designated personnel should write their initials and received date. The expiration date should also be circled. If an expiration date is not provided on the container, an anticipated expiration date should be written. The container should be properly stored according to the material safety data sheets (MSDS). A copy of the MSDS should be placed in the MSDS laboratory notebook replacing an out-dated copy if present. At the time of opening a container for initial use, the date opened should be recorded and initialed on the container.

Logbooks are needed for preparation of standards or solutions such as extractants. Entries in the logbook should include the following.

1. Time and date of preparation
2. Receptacle container for the solution being prepared
3. Name, amount and lot of chemicals used
4. Order of reagent addition during preparation
5. Required measurements (for example, initial pH for buffer solutions)
6. Associated soil samples analyzed
7. Chemist's initials

Careful attention should be given to chemical expiration dates on the container and how long the solution being prepared will be used. Any unused chemical or reagent removed from its original container should be discarded and not returned back to the original container.

Chemist Notebooks and Laboratory Records

Records in a chemist's notebook or a laboratory logbook provide a historical reproduction of activities in the laboratory. Without documentation, a chemist may have difficulty remembering specific actions taken that could help troubleshoot a problem. The guidelines below should be followed when documenting records.

1. Provide a historical reproduction of events.
2. Make entries in a permanent manner with blue or black ink. Do not use a pencil.
3. Be accurate, legible, and objective.
4. Entries should be recorded immediately after performing the action.
5. Follow an established retention policy for keeping the notebooks.
6. Protect and maintain integrity and security of data.
7. Record any modification in a procedure.
8. Contain information on all calculations to the final result if needed.
9. Document levels of review.

Errors will occasionally occur when performing or documenting various tasks. Guidelines to follow when recording these errors are shown below.

1. Draw a single line through the error so the original entry remains legible.
2. Write the correction adjacent to the error.
3. Never use white-out.
4. Initial and date changes made.
5. Offer a reason for the change if confusion is likely.
6. Add revisions to electronic files if they exist.

Labware and Glassware

In many high volume laboratories, plasticware has replaced glassware vessels. Plasticware is affordable, usually unbreakable, and can often be used without contamination if properly cleaned. For some tasks, disposable plasticware can offer an economical advantage compared to washing. For example, disposable paper or plastic cups can be used with automated pH instruments. Some metal contaminants, primarily zinc, can be present in high volume disposable plastic manufactured products. Laboratories should purchase these supplies in sufficient quantities to reduce the number of manufacture production runs or lots. Prior to use of the new lot, multiple blank extractions should be conducted and compared to existing inventory.

Most plastic vials used in daily extractions can be configured in a grouping of ten to twelve bottles that will allow for ease of handling as well as washing. Automated washing stations allow for grouped bottles to be thoroughly washed efficiently. An effective apparatus is a manifold with nozzles that bottles rest upon while water is controlled by a foot-pedal for washing the bottles. Three levels of rinsing are made with the first two being with tap water and the last one being made with deionized or distilled water (DI water). Filtering devices can be washed similarly at a sink. It is very important to wash labware before it becomes dry.

When glassware is involved, scrub to remove any residue. Soak and wash with phosphate-free detergent and triple rinse. If standards are prepared in volumetric flasks, the flasks should be soaked in a 0.1 M HCl solution for a few hours then rinsed thoroughly with DI water.

Volumetric pipettes can be washed using standard pipette washers and should be washed after each use. These should also be soaked in dilute HCl (not to exceed 1 M) prior to washing. All pipettes should be thoroughly dried prior to use.

Store any labware not in use in a clean environment as dust-free as possible. When possible, closed cabinets or drawers are best. Any new labware or labware removed from long-term storage should be thoroughly washed prior to use.

Daily Traceability of Samples and Laboratory Processes

The importance of records in logbooks and laboratory notebooks has been previously mentioned. Documenting chain of events for the progression of various steps of receiving, handling, and analyzing a sample is extremely beneficial. For each sample, it is important to know who performed each step, where solutions were attained, what instruments were used, and when each task was performed. This information is of tremendous assistance for resolving problems with errors in laboratory data. Table 2 lists guidelines for sample traceability for various laboratory processes.

*Table 2. Guidelines in tracking laboratory processes.*

| Lab Process | Task Conducted | Method of Record |
|---|---|---|
| Receiving samples | Assign lab ID to sample box/bag.<br>Enter lab ID on information sheet.<br>Designate position for QC samples.<br>Record the following:<br>Client name and shipment information<br>Date and time of arrival<br>Method of shipment<br>Condition of samples<br>Location of storage if needed<br>Special considerations if quarantine needed | Soil receiving logbook<br>Bar code scanner<br>Hardcopy submittal forms<br>Laboratory Information Management System (LIMS) |
| Assign analysis | Organize samples into sets for analysis.<br>Assign technician and due date. | LIMS |
| Grind soil | Grind soil samples to less than 2 mm.<br>Record technician, date, and time of process. | Grinding logbook |
| Measure soil | Label containers receiving soil with lab IDs.<br>Scoop or weigh samples and QCs. | Sample Prep logbook |
| Extraction | Add extractant to samples and shake.<br>Filter and collect fitrate for analysis. | Extraction logbook |
| ICP-AES analysis | Assign samples to instrument. | ICP-AES logbook or LIMS |
| pH / buffer pH | Add water and buffer.<br>Read pH and soil-buffer pH. | Data logger in pH meter, electrode logbook, or LIMS |

Training and Error Prevention

Standard operating procedures are excellent tools to train new employees and refresh experienced chemists. Training offered by instrument and chemical manufacturers is valuable as well. The QA/QC officer or laboratory manager should maintain a logbook documenting all training to ensure existing and new staff are fully aware of all laboratory procedures, changes in methodology, and instrumentation operation.

An important part of quality in laboratory work is having employees who are not only prepared with good technical skills, but also prepared with a good mental attitude. Prior knowledge of busy times of the year are important so employees are ready for the rigors of handling a large number of samples in a timely manner. Emergencies and illnesses will occur and employees may be absent unexpectedly. Cross-training is beneficial to ensure samples will be analyzed in a timely manner during employee absences. Cross-training is also beneficial so employees can be cycled to different tasks to avoid monotony associated with continuously doing the same laboratory test for long periods of time.

No matter how diligent employees are, errors will occur. Anticipating errors through a thorough understanding of specific work flow processes is extremely important. Data should be reviewed by technicians generating the data and further reviewed by supervisors or QA officers to prevent erroneous data from being reported to clients. A conscientious technician can improve efficiency by recognizing poor quality data as it is being generated and correcting the error before submitting data to supervisors or QA officers.

Some common errors that may occur in soil testing laboratories are shown in Table 3. Details are given for the laboratory location, actual error, result if uncorrected, and actions that can be taken to minimize the potential for the error to reoccur.

## Assessment of Data Quality

The product of soil test laboratories is data. The quality of that data needs to be assessed before it is delivered to the client. The previous section described various QC measures to take in the laboratory for producing quality data. Another important QC action taken is a statistical evaluation of data generated to determine if any errors occurred in laboratory analyses. Data are evaluated on soil samples with known test results that are tested along with client samples. Data can also be evaluated on the analytical process conducted without a soil sample. These data are useful for evaluating detection limits or background contamination of the analyte in the analytical procedure.

Accuracy and Precision

Accuracy and precision are two words used when qualifying data. Accuracy is a measure of how close an analytical result is to the "real" concentration of the analyte. Precision is a measure of how consistent the analytical result is with repeated measurements. An analogy to shooting a target is often made to describe accuracy and precision with the bull's eye considered the "real" concentration of the analyte. If you shoot a gun five times directly into the center of the bull's eye on a target, you have very accurate and precise results. If you shoot a gun five times in the same location on the target but it is not in the bull's eye, you have very precise results but they are not accurate. Precision measures the scatter of data around a mean. Tightly clustered data indicates great precision but does not necessarily indicate great accuracy. When data are highly

*Table 3. Some potential errors that can occur in soil testing with resultant outcomes and follow-up actions.*

| Location | Error | Outcome | Action |
|---|---|---|---|
| Receiving | Misalignment of samples<br>Incorrect lab numbers on the client information sheet | Client receives incorrect sample results and recommendations | Carefully review work after assigning lab numbers to sample box/bag and information sheets. |
| Receiving | Not placing a control sample in a group of samples | Loss of QA/QC for the group of samples involved and uncertainty on data quality | Carefully review work when logging in samples. |
| Grinding | Placing samples out of sequence as samples are ground | Lab numbers and samples are out of sequence so client will receive incorrect data and recommendations | Work in verified order without deviation and review lab number order before and after grinding a set of samples. |
| Grinding | Samples not sufficiently dry so soil adheres to the grinder and contaminates samples | Inaccurate results for samples | Thoroughly check samples for dryness. Dry further if necessary. Clean grinder in between samples if needed. |
| Measurement | Using the wrong size scoop for an analysis | Inaccurate results for samples | Paint handles of similar size scoops the same color and store scoops of different sizes apart from one another. |

*Table 3 (continued). Some potential errors that can occur in soil testing with resultant outcomes and follow-up actions.*

| Location | Error | Outcome | Action |
|---|---|---|---|
| Measurement | Samples scooped out of order | Client receives incorrect sample results and recommendations | Train employees on systematic sample scooping and make sure control samples are scooped in correct positions. Control samples should be scooped in the order they occur, not all at one time. |
| Soil Extraction | Filtrate is poured out of sequence | Client receives incorrect sample results and recommendations | Label each extraction rack and filtering apparatus with a color code and beginning lab number. |
| ICP-AES analysis | Instrument output displays near zero concentrations for various elements due to blockage in autosampler tubing/nebulizer assembly | Client receives incorrect sample results and recommendations | Review data prior to importing into LIMS. Inspect autosampler tubing and nebulizer assembly frequently. Look for failure in QC samples and loss of sensitivity during recalibrations. Remove blockages. |

precise but inaccurate, random error is small but there is some systematic error in the analytical procedure resulting in inaccurate results.

Samples with known concentrations are placed in a group of client samples to evaluate the precision of analytical results. These samples are referred to as QC, control, check, or internal reference samples. Since results for these samples are known, any deviation of a result from a known result is an indication that some error occurred in the laboratory process. These control samples only measure precision of analytical results because the "real" concentration of the analyte is not known. Certified reference samples can be obtained with actual concentrations to provide a measure of accuracy. However, these samples are expensive and only provide a certified analysis for total concentration of analytes in soil or plant material. Certified reference samples are not available for soil analyte concentrations determined with various extractants common in soil test laboratories.

Control samples for analysis by soil test extractants and soil pH are generally formulated within the laboratory from soil within the region. Another option for control samples is to purchase bulk soil from a proficiency testing program (NAPT, 2013; ALP, 2013). These soils have a median concentration determined from testing conducted at several soil testing laboratories. Although the median concentration is not a "real" concentration that is obtained with a certified reference sample, it is a concentration that should closely approximate a "real" concentration so accuracy of a laboratory procedure can be assessed. The chance of the median value approximating a "real" concentration increases with an increase in the number of laboratories providing a value for the median.

The following guidelines should be reviewed when selecting control samples for use in a soil testing laboratory.

1. Select soils similar in chemistry, texture, and organic matter content to those analyzed for clients.
2. Strive for several different check samples that encompass the range of results typically observed.

If the control sample is prepared by the laboratory, the following guidelines apply for preparing the sample.

1. Dry, grind and screen soil to a particle size less than 2 mm.
2. Thoroughly mix the sample to ensure sample homogeneity. Mixing large amounts of soil to ensure homogeneity is not a trivial task. An affordable and effective method of mixing starts with raking a thin layer of soil on a clean tarp. The sample is mixed by pulling one corner of the tarp all the way to the opposing corner. Soil is again raked to a thin layer over the whole tarp. Another corner of the tarp is pulled to an opposing corner followed by raking soil over the entire tarp. This process is conducted several times to ensure good mixing. Another method of mixing is through use of a cement mixer.
3. Depending on the total volume of soil processed, subsample the thoroughly mixed sample about 30 times using a random sampling protocol. The resulting subsamples will be used to determine the variability of different soil parameters and whether additional mixing of the

bulk sample is required. Analyses of the collected subsamples should follow the established SOPs for the laboratory. It is recommended that the set of subsamples be further subdivided into three smaller groups and analyzed during different analysis runs on different days.

4. Accepted tolerance in variability for different soil parameters is dictated by the quality assurance objectives for each laboratory. If analyses of the subsamples fall outside of the desired tolerance limits, further mixing of the designated soil is recommended.
5. Once the desired tolerance limits are reached for the bulk soil, a further assessment of precision and accuracy for the desired soil parameters can be obtained by conducting an interlaboratory comparison study with soil testing laboratories using similar SOPs.

Variability in measurements will come from inherent variability within the samples due to variability in sample preparation equipment (e.g., scoops and pumps), technician technique, and analytical instrumentation. If a laboratory has multiple instruments of the same type, then the check samples should be analyzed on each instrument to compare results. Bias between different technicians can be investigated by having check samples repeatedly analyzed by an individual technician and comparing the results to a mean from all laboratory personnel. If an individual's results are consistently greater than or less than the mean, there is some systematic error in that individual's technique.

Use of check samples to monitor overall laboratory performance is achieved using control charts. It is assumed that the distribution of values for a given soil parameter in the check soil approximates a normal distribution that can be adequately described by the mean (X) and standard deviation (s). Limits on performance are set as multiples of the standard deviation. Two times the standard deviation (2s) is where 95% of all observations should fall with a normal distribution. Three times the standard deviation (3s) is where 99% of all observations should fall with a normal distribution. The 2s range is commonly used as a warning limit signifying something may be wrong with data if it falls outside of this range. The 3s range is commonly used as a control limit where data falling outside of the range indicate a high likelihood of something in the measuring process being out of control. Typical shorthand notation for these designated limits in control charts are shown below.

Upper Warning Limit (UWL) = X + 2s
Lower Warning Limits (LWL) = X – 2s

Upper Control Limit (UCL) = X + 3s
Lower Control Limits (LCL) = X – 3s

The values for the mean and standard deviation used to establish a control chart are obtained from repeated analyses on one sample as shown in Table 4 for Mehlich-3 P. The calculated analytical limits (LWL, UWL, UCL, and LCL) are then plotted as horizontal lines on a quality control chart known as an X-chart. Fig. 1 shows an X-chart with analytical limits and data points from Table 4.

It is common for the control limits of control charts to be modified with time during the initial use of a new check sample, adoption of new analytical protocols, or modifications in SOPs. Changes in control limits reflect a changing degree of confidence in the results as laboratory staff become familiar with modified or new SOPs, or a better estimate of sample

*Table 4. Twenty analysis runs of Mehlich-3 P on a check soil sample used to establish an X-chart.*

| Run | P mg dm$^{-3}$ | Run | P mg dm$^{-3}$ | Run | P mg dm$^{-3}$ | Run | P mg dm$^{-3}$ | Run | P mg dm$^{-3}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.63 | 5 | 7.75 | 9 | 7.55 | 13 | 8.15 | 17 | 7.64 |
| 2 | 8.13 | 6 | 7.92 | 10 | 7.41 | 14 | 7.96 | 18 | 7.93 |
| 3 | 7.48 | 7 | 7.55 | 11 | 7.86 | 15 | 7.71 | 19 | 7.77 |
| 4 | 7.92 | 8 | 7.42 | 12 | 7.56 | 16 | 8.18 | 20 | 8.03 |
| Mean = 7.83; standard deviation = 0.31; number of samples = 20 | | | | | | | | | |
| LWL = 7.21; UWL = 8.45; LCL = 6.90; UCL = 8.76 | | | | | | | | | |

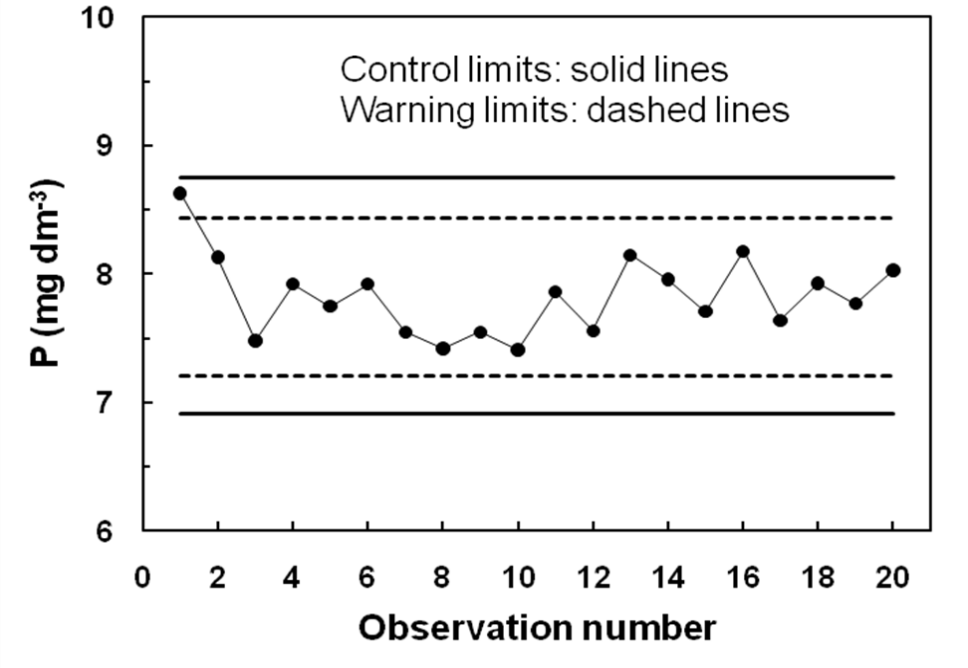


*Fig. 1. Phosphorus data from Table 1 plotted as a quality control X-chart.*

variance is obtained over time. After several months, depending on the frequency of analyses of the check sample for a given soil parameter, control limits should be finalized.

Control charts serve a variety of purposes. The most important purpose is to check daily laboratory analyses. If a check sample falls outside of established warning or control limits for a given soil parameter or analyte, a set of decision criteria needs to be established for dealing with such occurrences. The goal of the decision criteria is to determine the cause of the observed value and corrective action necessary to bring a given measurement process back into control. The first set of criteria needing developed is determining when corrective actions are necessary. Possible considerations in determining when a corrective action is necessary are shown below.

1. A single occurrence may not warrant action. Since the limits are set on 95% and 99% probabilities of data falling within the limits, there is some probability that a result for a check sample will fall outside the limits.
2. Is there a consistent pattern in values falling outside of the warning limits? More than 2 occurrences across several days would suggest a failure in the measurement process.
3. Are all analytes involved failing across several control charts, or is only one analyte demonstrating a problem? All analytes exceeding the limits indicates a consistent error in the measuring process affecting all analytes.
4. There are no observations that fall above or below the warning or control limits, but the overall pattern in the control chart is not randomly distributed around the mean value. This pattern indicates some consistent change occurred in the measurement process.

Corrective actions are more necessary when data exceed control limits (3s) rather than warning limits (2s). As the name implies, data outside the warning limits serves as a warning that something may be wrong. When data is out of control limits, action is usually required to correct the data. Once it is determined that corrective action is necessary, the next step is to determine the possible cause for the data to exceed the control limits. There are a variety of possible reasons for failure during the measurement process. Data out of control limits may be due to an individual technician's technique in the measurement process or a specific instrument. If the problem is not isolated to one instrument or technician, then a thorough review of the steps outlined within a given SOP needs to be taken and perhaps further tests conducted. It is important to assess the current state of the check sample and to make sure the sample is remaining homogeneous. If the sample is becoming segregated relative to particle size, this may affect the data obtained on the sample. If the check sample is segregated, new control limits may need to be established. Whatever the cause for data to be outside the control limits, it is imperative that a thorough discussion and investigation among supervisors and staff occur to determine where the problem exists.

Final decision criteria need to be established on whether to accept the data generated when the control failure was detected. One option is to repeat all analyses subsequent to when the results for the check sample were last in control. This may or may not be possible due to sample size as well as cost restrictions. If the analyses cannot be repeated, then suitable criteria are needed on how the data will be flagged and eventually reported. Results from more than one set of control procedures may be used in making a decision.

The number of check samples to use in as set of routine samples is a laboratory management decision. There is a monetary cost associated with check samples, both in time and analytical overhead. In most soil labs, a check sample is analyzed for every 20 to 30 samples. The checks are inserted and analyzed as part of the daily sample stream. The check samples along with the mean and standard deviation of analyses can be known or unknown to the laboratory staff. An advantage of the check samples and data being known is that a technician can immediately detect if measurements are out of control and decide on whether retesting is necessary. If the location of the check sample is known but the mean and standard deviation is unknown to the technician, this is a blind control sample. For these control samples, a laboratory manager or QA officer is required to make a decision on what actions to take if data fall outside of warning or control limits. Blind control samples have an advantage of ensuring data integrity where the individual checking the data is different than the individual conducting the analysis. If the location of a check sample and the data on mean and standard deviation are unknown to the technician, this is

a double-blind control sample. The advantage of double-blind control samples is that the sample is ensured to be treated exactly like all other samples going through the laboratory since the technician is unaware of the control samples' existence.

Check samples are used to monitor analytical control on a daily basis. It is also advisable for laboratories to participate in proficiency testing programs where sample results can be compared across several labs. These programs involve analysis of various soil samples on a regular time frame throughout the year. Typically, multiple labs using various methods are involved and data from labs are statistically analyzed to estimate a "true" value for analytes from the median or mean of all results. This approach is excellent as long as the population of participating labs for a given method is not too small. Proficiency programs are affordable and utilized by many private and public labs across North America as well as internationally. Two proficiency soil testing programs available are North American Proficiency Testing (NAPT) and Agricultural Laboratory Proficiency (ALP) programs (NAPT, 2013; ALP, 2013).

Control charts provide a measure of precision in analyses for the check samples over relatively long periods of time. To the extent that check samples resemble the sample matrix of grower samples, it is assumed that the precision demonstrated in the control charts reflect the level of precision on a grower's sample. Testing samples in duplicate can also be done to provide a separate check on analysis precision directly on growers' samples.

A type of control chart using duplicate analyses of sample unknowns is a range chart (R-chart) where differences between duplicates samples analyzed in a set of samples are expressed as absolute differences or absolute relative differences. Duplicate analyses of Mehlich-3 K for a variety of samples are shown in Table 5. The absolute relative difference percent (RD%) is calculated as:

$$RD\% = 100 \mid [(X_{rep1} - X_{rep2})] \div [(X_{rep1} + X_{rep2})/ 2)] \mid$$

As with the quality control X-chart, the mean and standard deviation of the RD% values are calculated and a control limit is set as a multiple of the standard deviation. The warning and control limits are calculated as the mean RD% + 2s and mean RD% + 3s, respectively, with respective values of 18.6% and 24.4%. For the example in Table 5, results for K are under statistical control for precision since the calculated RD% values for each sample falls below the control limit.

The RD% values of the 11 samples in Table 5 are plotted as a quality control R-chart in Fig. 2 with the warning and control limits. The data are from duplicate analyses of grower samples. Control samples can be evaluated in a similar fashion if they are analyzed in pairs.

An R-chart can also consider absolute difference between duplicate measurements: $\mid X_{rep1} - X_{rep2} \mid$. The mean of the absolute average differences (R) is determined and control limits are calculated as follows.

Warning Limit (UWL) = 2.512 x R for a 95% confidence interval
Control Limit (UCL) = 3.267 x R for a 99% confidence interval

The coefficients of 2.512 and 3.267 are values used for 2 replications. The coefficients for a subset with more than 2 replications decrease with increasing number of replications (Kume, 1985).

*Table 5. Eleven analysis runs of duplicate sets of Mehlich-3 K soil used to establish a quality control R-chart.*

| Sample | K (Rep 1) | K (Rep 2) | RD% |
|---|---|---|---|
| | ---------- mg $dm^{-3}$ ---------- | | |
| 1 | 8.6 | 8.1 | 5.5 |
| 2 | 9.8 | 9.7 | 1.4 |
| 3 | 5.5 | 4.9 | 4.8 |
| 4 | 21.7 | 19.5 | 11.4 |
| 5 | 17.4 | 16.8 | 1.5 |
| 6 | 59.8 | 63.2 | 10.4 |
| 7 | 5.9 | 5.7 | 3.0 |
| 8 | 7.9 | 7.5 | 7.1 |
| 9 | 4.1 | 3.4 | 18.7 |
| 10 | 3.5 | 3.4 | 1.1 |
| 11 | 16.1 | 15.0 | 13.7 |
| RD% mean = 7.13%, standard deviation = 5.76%, number of samples = 11<br>Warning limit = RD% + 2s = 18.6%; Control limit = RD% mean + 3s = 24.4% | | | |

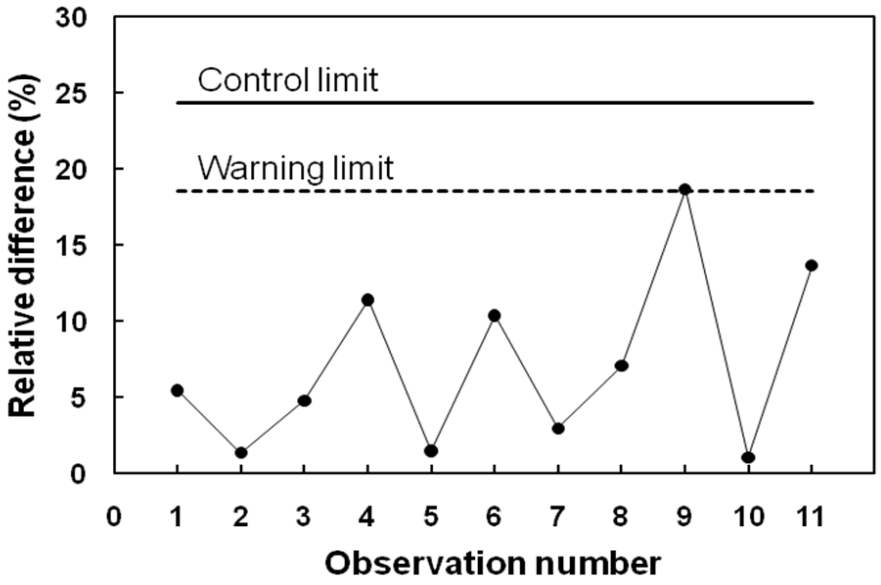


*Fig. 2. Potassium data in Table 2 plotted on a quality control R-chart.*

Analytical Limits in Measurements

There are limits in analytical chemistry that need to be recognized to ensure values reported to clients are real. Four of these limits discussed below are 1) limit of detection (LOD), 2) limit of quantification (LOQ), 3) limit of calibration (LOC), and 4) quantitative resolution (QR).

LOD: The minimum concentration at which the analyte can be reported with a 99% certainty that the analyte exists at a concentration other than zero. Limit of detection can have units of mg $L^{-1}$ if referring to concentration in a soil extract. For concentration in soil, mg $dm^{-3}$ or mg $kg^{-1}$ is used. Method detection limit (MDL) is another commonly used value similar to LOD.

LOQ: The lowest concentration in which an analyte can be quantified and reported with certainty. Limit of quantitation is greater than LOD. As with LOD, LOQ has units of mg $L^{-1}$ if referring to concentration in a soil extract. For concentration in soil, mg $dm^{-3}$ or mg $kg^{-1}$ is used. Method reporting limit (MRL) is another commonly used value similar to LOQ.

LOC: The highest concentration of a range of concentrations in standards used to calibrate the instrument. The concentration range from the LOQ to the LOC is the working range of measureable values of the analyte.

QR: The minimum concentration change in an analyte that can be quantified from an analytical method considering sensitivity of the instrument response to increasing analyte concentration and uncertainty of the instrument response.

*LOD and LOQ*

The difference between LOD and LOQ can be likened to a situation where you would listen to someone talk with a lot of noise in the surrounding environment. Consider the sound of the individual's voice to be analogous to the presence of an analyte in a sample. If there is so much surrounding noise and you cannot hear the voice at all, the voice level is below the limit of detection. If you can hear the voice but cannot understand the words being spoken, the voice level is detected so you know it exists but you cannot quantify the content of the conversation. Not until the voice is loud enough, or the surrounding noise is low enough, would you be able to discern words and sentences from the voice which would be above the LOQ. If an analyte has a concentration between the LOD and LOQ, you can state with confidence that the analyte exists in the sample. However, you cannot state with confidence the concentration of the analyte until the concentration is above the LOQ.

The LOD and LOQ of an analyte are determined by obtaining the instrument signal on several replicate runs of the blank used to calibrate the instrument. The LOD is an analyte concentration that corresponds to 3 times the standard deviation of the instrument signal from the replicate runs. The LOQ is an analyte concentration that corresponds to 10 times the standard deviation of the instrument signal from the replicate runs. To determine the LOD or LOQ, first obtain the average ($x_b$) and standard deviation ($s_b$) of 10 signal readings of the blank calibration standard. The blank calibration standard is a solution of the soil extractant with no analytes. For ICP-AES, the signal reading is an intensity count. Next, obtain the slope (m) and intercept (b) of the calibration curve which plots signal reading versus concentration in solution as mg $L^{-1}$. The parameters are entered into the following equations to determine LOD and LOQ in units of mg $L^{-1}$ in soil extract.

$$LOD = (x_{bi} - b + 3\ s_{bi}) / m$$

$$LOQ = (x_{bi} - b + 10\ s_{bi}) / m$$

To determine LOD and LOQ for concentration of the analyte in soil, use the appropriate conversion factor to obtain a soil concentration from the unit of mg $L^{-1}$. For example, a Mehlich-3 test on soil has 2 g soil extracted with 20 mL of Mehlich-3 solution. The LOQ in soil in units of mg $kg^{-1}$ would be 10 times the LOQ in the extract in units of mg $L^{-1}$.

If a concentration is below a LOQ, the value reported should be "< LOQ". If Mehlich-3 P LOQ were 1 mg $kg^{-1}$ and the instrument reading indicated a concentration of 0.5 mg $kg^{-1}$, < 1 mg $kg^{-1}$ would be the more accurate result to report. With regards to fertilizer P recommendation, there would be no difference between 0.5 and 1 mg $kg^{-1}$. Thus, soil test laboratories rarely have the need to assess LOQ for plant macronutrients. However, this may not be the case for plant micronutrients, heavy metals, or herbicide analysis. Limit of quantitation should be determined for test procedures determining low concentrations of analytes that have practical consequences in recommendations so there is confidence that the reported concentrations are real.

Instrument manufacturers often provide specified detection limits for a given analyte. These instrumental LODs are typically lower than what can be attained in soil extracts. The instrumental LODs are obtained with a simple acid matrix where the analyte may be the only element existing in solution. Soil extracts contain many analytes with a complex matrix of acids and chelates that can affect LODs.

The values for method detection limit (MDL) and method quantification limit (MQL) describe the LOD and LOQ, respectively, when errors are taken into account that occur in the process of preparing the sample being analyzed by the instrument. These errors may occur in extraction, digestion, or dilution processes. The MDL and MQL are determined by introducing the analyte in a blank sample early in the method procedure at a concentration estimated to be near the LOD or LOQ. Several replicates of these samples are prepared and analyzed to determine MQL and MRL (Taylor, 1987). The method of determining MDL and MQL is not straightforward. The extra time and resources required to determine MDL or MQL may not be warranted since MDL and MQL are often very close to LOD and LOQ and the significance of interpreting most data at very low concentrations in soil test laboratories is not consequential.

When determining analytes at low concentrations, it is important to determine if glassware, plasticware, or filter paper are contamination sources for the analyte in the method procedure. Method blanks are used for this determination. Method blanks are different from calibration blanks used to calibrate an instrument. A method blank is a solution from a soil extraction method that has experienced every step in a method procedure with the exception that soil was not placed into the process. For a soil extraction, the soil test extractant would be placed into a container without soil, shaken for a prescribed period of time, and filtered to collect a filtrate for analysis. If the concentration in the filtrate is greater than the LOD, the analyte has entered the method procedure as a contaminant. Concentration in method blanks is most important in measurements of low concentrations that occur with micronutrient, heavy metal, or herbicide analysis of soil extracts. Methods should be evaluated with blanks to determine if significant contributions occur from the testing procedure. If the contamination is significant and the source cannot be eliminated, the concentration of the method blanks should be subtracted from the sample concentrations. An alternative to subtracting concentrations from a method blank is to prepare a calibration blank and calibration standards from a method blank. The background

concentration of analyte would thus be neglected in the calibration of the instrument. This alternative may be useful for solution samples submitted by clients with an unknown or complex matrix.

*LOC*

Instruments rely on a calibration of instrument response to analyte concentrations in standards. The range of concentrations from the LOQ to the highest concentration standard is the dynamic range of concentrations that can be determined in unknown samples. If a sample concentration is greater than the highest concentration in the dynamic range, the analyst should be wary of the result because it is outside of the range of instrument calibration. If the calibration response is linear, there is no certainty the response remains linear at concentrations greater than the dynamic range. If the response beyond the dynamic range becomes quadratic with decreasing instrument sensitivity and it is assumed to remain linear, the determined sample concentration will be lower than the actual concentration. To ensure sample concentrations are being detected within a dynamic range, the unknown can be diluted with the dilution factor taken into account to report final concentrations. Another alternative is to add a calibration standard with a greater concentration. Instruments often respond to analytes at higher concentration with less and less sensitivity. The instrument may detect the analyte at higher concentration but detection may occur with less sensitivity requiring a quadratic calibration curve.

*QR*

The number of significant figures that should be reported from a chemical analysis depends on the sensitivity of an instrument's response to an increasing concentration of the analyte. Sensitivity is the slope of a calibration curve and can be mathematically shown as follows:

$$S = \Delta R / \Delta C$$

where $\Delta R$ is the change in instrument response and $\Delta C$ is the change in analyte concentration.

There is uncertainty in an instrument's response that can be quantified with repeated analysis of a calibration standard. This uncertainty divided by the sensitivity of the instrument yields the uncertainty in the analyte concentration. The analyte concentration uncertainty is the minimum change in concentration producing a measurable change in instrument response. This value has been termed QR (Pardue, 1997) and is mathematically expressed as follows:

$$QR = e / S$$

where e is the uncertainty in the instrument's response which can be determined as the standard deviation from repeated analyses of a calibration standard.

## Summary

The primary goal of a soil test laboratory is to provide reliable, consistent and valid data. These data can be used to generate soil fertility recommendations for farmers and consultants making management decisions or possibly used in environmental regulation. Laboratory QC and management's commitment to QA are vital in this process.

The laboratory's QC guidance for data generation is a set of well written and followed SOPs that provide sufficient detail for a procedure to be completed without personal judgment or preference. In order to do this successfully, the chemist must utilize quality chemicals, standards, and solutions and use optimized instruments and equipment to achieve desired results. Potential contamination from labware must be safeguarded. Archiving records of detail (logbooks, chemist notebooks, lab records, etc) to verify or document various steps in the generation of results for traceability purposes is essential. To the extent possible, training all chemists in all phases of laboratory procedures is paramount to minimize errors and to maintain a highly functional, efficient lab, even when staff absenteeism occurs.

Before releasing data to clients, a careful assessment of the data is required for QA. Precision is the consistency of attaining statistically similar analytical values over time and is evaluated by analysis of check samples in daily runs of client samples. Evaluation of the check sample data for precision is achieved through daily graphs of statistical upper and lower warning and control limits for an analyte. Data under control without bias are usually randomly distributed about the mean within the warning limits. If data are outside of control limits, decision criteria are essential to identify the cause of the outlying value and if action is needed to bring the data under control. Precision can also be measured by duplicate analysis of client samples. Accuracy is the measure of how close an analytical result is to the real analytical value and can be assessed by analyzing standard reference samples. However, standard reference samples do not exist for assessing accuracy with various soil test extractants commonly used in soil test laboratories. Accuracy can be approximated with consensus values obtained on one sample analyzed by several different laboratories as occurs in proficiency testing programs. Two proficiency programs in soil testing are NAPT and ALP and all soil test laboratories are encouraged to participate in one of these programs.

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# *Unit 3*

# Soil pH and Lime Requirement

*Chapter 3.1*

# Introduction to Soil pH and Lime Requirement

*F.J. Sikora*

Soil pH is a measure of hydrogen activity and provides information on the acidity or alkalinity level in soil. Most crops have optimum soil-water pH ranges between 6 and 7. When soil-water pH is below a critical level, lime application is required to increase pH. To determine lime required to increase pH to some target value, a test is required to assess the acidity held onto soil colloids. Methods using buffer solutions have been developed for quantifying this acidity. The Shoemaker, McLean, and Pratt (SMP) (Shoemaker et al., 1961) and the Adams-Evans (Adams and Evans, 1962) buffers were developed in the early 1960s. The SMP buffer was developed for silt loam soils in Ohio with appreciable exchangeable Al. The Adams-Evans buffer was developed in Alabama for soils with lower cation-exchange capacity, pH buffer capacity, and lime requirement. The Mehlich buffer (Mehlich, 1976) was developed in 1976 with a primary focus of reducing phytotoxic levels of aluminum to a nontoxic level rather than increasing soil pH to a target value. These buffer solutions have different pH and pH buffer capacities. When added to soil, each buffer reacts with soil acidity and a soil-buffer pH is measured. Soil-buffer pH is correlated to data from soil-lime incubation studies to determine lime required to increase soil-water pH to a target value.

There are chemicals hazardous to human health in the buffer solutions which require special precautions be followed for hazardous waste disposal. The hazardous chemicals include p-nitrophenol in Adams-Evans buffer, p-nitrophenol and chromate in SMP buffer, and barium in Mehlich buffer. The presence of hazardous chemicals in the buffers has resulted in several investigations for alternative methods to determine lime requirement. Auburn University developed a modified Adams-Evans buffer containing phosphate rather than p-nitrophenol to buffer pH (Huluka, 2004). The University of Kentucky developed the Sikora buffer to replace SMP buffer by using imidazole and MES rather than p-nitrophenol and chromate (Sikora, 2006). The University of Georgia developed an approach of adding $Ca(OH)_2$ to soil after an initial pH measurement in 0.01 *M* $CaCl_2$ to determine the pH buffer capacity of the soil (Kissel et al., 2007). Clemson University developed the Moore-Sikora buffer which acts like the Adams-Evans buffer by replacing p-nitrophenol with MES and MOPS (Sikora and Moore, 2008). The University of Maine developed a modified Mehlich buffer which replaces the barium in the buffer with calcium (Hoskins and Erich, 2008).

When determining lime requirement from a soil-buffer pH measurement, calibration is required on a set of soils for which lime recommendation will be made from the soil-buffer pH measurement. For the Adams-Evans buffer and subsequent buffers designed to replace Adams-Evans buffer, calibrations were performed by obtaining the relationship between soil pH and the percent acid saturation of the cation exchange capacity (Adams and Evans, 1962; Huluka, 2004). The soil-buffer pH determines the amount of exchangeable acidity needing neutralized between the existing soil pH and the desired soil pH using the calibrated relationship between soil pH and percent acid saturation. For SMP buffer, calibrations were conducted by relating soil-buffer pH to the amount of lime needed in laboratory incubation studies (Shoemaker, 1958; Godsey et al., 2007). The Sikora buffer produces the same soil-buffer pH as the SMP buffer so original

calibration data with SMP buffer are valid for soil-buffer pH using the Sikora buffer. For modified Mehlich buffer, calibrations were also conducted with laboratory incubation of lime and soil (Hoskins and Erich, 2008; Wolf et al., 2008).

Single soil-buffer pH measurements require calibration for lime recommendation. If multiple pH measurements are used to determine pH buffer capacity of each soil tested, calibration to determine amount of lime to reach a target pH is not required. The single addition $Ca(OH)_2$ and Sikora-2 buffer methods provide information on pH buffer capacity of soil. In both methods, an initial soil pH is determined in a salt solution using 0.01 *M* $CaCl_2$ with single addition $Ca(OH)_2$ or 1 *M* KCl with Sikora-2 buffer. A second pH is determined after adding $Ca(OH)_2$ or Sikora-2 buffer. Since the second pH is at the same ionic strength as the initial pH, two points are obtained that can be used to estimate a lime response curve of pH versus alkalki addition whose reciprocal slope defines the pH buffer capacity of the soil. Some calibration to field application of lime is required to compensate for short reaction times in lab measurements and incomplete reaction of alkali in the soil.

The following chapters provide details on the original methods using Adams-Evans, SMP, and Mehlich buffers along with each of the alternative methods that eliminates hazardous waste (modified Adams-Evans, Moore-Sikora, Sikora, and modified Mehlich), or eliminates hazardous waste and provides information on pH buffer capacity of soil (single addition $Ca(OH)_2$ and Sikora-2).

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*Chapter 3.2*

# Soil pH

*F.J. Sikora and D.E. Kissel*

## Application and Principle

Soil pH is an important measurement to assess potential availability of beneficial nutrients and toxic elements to plants. Soil pH is determined with an $H^+$ ion-selective glass electrode and is a measurement that can be performed for all soils.

A common method of measuring soil pH is performed by placing a glass electrode in a mixture of soil and deionized water. Most plants grow optimally with a soil-water pH from 5.7 to 7.0. Various modifications exist for determining soil pH. The most common ratio used for soil-water pH is 1:1 soil/water. Some laboratories measure pH in a 1:2 ratio of soil/water to improve the fluidity of the slurry; particularly for soils with high organic matter and clay concentrations that can absorb a significant volume of water. Electrolyte solutions, such as 0.01 *M* $CaCl_2$ or 1 *M* KCl, can be added to soil rather than deionized water with the resultant pH referred to as salt pH. Use of electrolyte solutions avoids variable soil-water pH due to varying background salt levels in different soils and improves electrical conductivity in the electrical circuit for pH measurement (Miller and Kissel, 2010). Soil pH measurement in deionized water or 0.01 *M* $CaCl_2$ in 1:1 and 1:2 soil/solution ratios are official methods adopted by the Association of Official Analytical Chemists (Kalra, 1995; Kane, 2012). Automated instruments have begun to be popular for measuring soil pH since these instruments save labor costs and improve accuracy of measurements. This chapter presents the common measurement of soil pH in a 1:1 mixture of soil to deionized water along with modifications with electrolyte solutions, soil/solution ratio, and automated instruments.

## Equipment and Apparatus

1. Soil scoop with 10 or 20 $cm^3$ capacity if measuring soil by volume
2. Analytical balance with 0.1 g resolution if measuring soil by weight
3. Glass, plastic, or waxed-lined paper cups that are approximately 50 mL
4. Holding rack for cups
5. Volume dispensers for adding 10 to 20 mL of deionized water or electrolyte solution to soil
6. Manual pH meter or robotic pH analyzer
7. Glass pH electrode with an internal reference element or a separate reference electrode

## Reagents

1. *Deionized water*
2. *Standardization buffers at or near pH 4, 7, and 10*

3. *Calcium Chloride ($CaCl_2$), 0.01 M*: Weigh 1.47 g of $CaCl_2 \cdot 2H_2O$, transfer to a 1-L volumetric flask, and add approximately 800 mL of deionized water to dissolve. After dissolution is complete, bring volume to 1 L with deionized water.
4. *Potassium Chloride (KCl), 1 M*: Weigh 74.6 g of KCl, transfer to a 1-L volumetric flask, and add approximately 800 mL of deionized water to dissolve. After dissolution is complete, bring volume to 1 L with deionized water.

## Procedures

1. Measure 10 $cm^3$, 20 $cm^3$, 10 g, or 20 g of processed soil (dried, < 2 mm) and add it to a sample cup. Volume is measured with a soil sampling scoop. Weight can be measured with an analytical balance or estimated from a volume measurement assuming a density of the processed soil. For example, 20 $cm^3$ scoop measures 25 g assuming a density of 1.25 g $cm^{-3}$. Other volumes or weights can be measured as long as the volume of solution added in the next step conforms to the ratio of soil to solution.
2. Add solution according to the type of soil pH being determined.
   a. *1:1 soil/water pH*: Dispense a particular volume of water to soil that is equal to the volume or mass of soil.
   b. *1:1 soil/0.01 M $CaCl_2$ pH*: Dispense a particular volume of 0.01 *M* $CaCl_2$ to soil that is equal to the volume or mass of soil.
   c. *1:1 soil/1 M KCl pH*: Dispense a particular volume of 1 *M* KCl to soil that is equal to the volume or mass of soil.
   d. *1:2 soil/solution pH*: Dispense a particular volume of solution (water, 0.01 *M* $CaCl_2$, or 1 *M* KCl) to soil that is twice the volume or mass of soil.
3. Stir the soil and solution vigorously and allow slurry to sit from 15 min to 1 h before measuring pH.
4. Ensure room temperature is between 20 and 25$^oC$ before proceeding with pH measurement.
5. Calibrate pH meter with calibration buffers. For acid soils, perform a 2-point calibration with pH 4 and 7 buffers. For alkaline soils, perform a 3-point calibration with pH 4, 7, and 10 buffers or a 2-point calibration with pH 7 and 10 buffers. After calibration, measure pH in one of the calibration buffers. If the pH exceeds the calibration buffer pH by ± 0.05, recalibrate the pH meter. If measured pH continues to exceed expected value, replace the electrode.
6. Place electrode in the soil slurry to measure pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for automated instruments. Rinse the probes in deionized water before measuring the next sample.
7. If using an automated pH instrument, place samples in the instrument with software set to adjust the timing of electrode measurements. LabFit (2013) has a robotic pH instrument that measures pH approximately every second. Software controls the time an electrode is in a sample before beginning pH measurement (Sample delay time), the number of pH measurements to assess stability (Number of readings for stability), the pH variance to assess pH stability (pH stability), and the maximum time allowed for pH measurements to occur (Maximum sample time). Details on these parameters and their effects on pH measurements

*Table 1. Parameter settings for pH measurements on a LabFit robotic pH instrument at four University soil test laboratories.*

| | Clemson Univ. | Univ. of Kentucky | Univ. of Georgia | Virginia Tech |
|---|---|---|---|---|
| Type of soil pH measurement | 1:1 (v/v) soil/water | 1:1 (v/v) soil/1 *M* KCl | 1:1 (v/v) soil/ 0.01 *M* $CaCl_2$ | 1:1 (v/v) soil/water |
| Sample delay time (sec) | 15 | 5 | 16 | 5 |
| Number of readings for stability | 8 | 10 | 4 | 10 |
| pH stability | 0.01 | 0.02 | 0.03 | 0.02 |
| Maximum sample time (sec) | 60 | 60 | 30 | 90 |

can be found in Sikora et al. (2011). LabFit settings used by four University laboratories are shown in Table 1.

The LabFit instrument provides the option of rinsing or not rinsing electrodes between samples. Analyzing a set of samples is quicker when not rinsing electrodes, but there is a chance of sample carryover affecting measurements. The risk is greater when measuring soils with a wide range of soil textures and pH buffering capacities. For example, an acid sandy soil with low pH buffer capacity may have its measurement influenced by carryover of a previous clayey sample with high pH. It is best to set the instrument to rinse electrodes in between samples if soils being tested have considerable variability in soil textures and pH buffer capacities.

## Calculations

1. If measuring soil pH in 0.01 *M* $CaCl_2$ or 1 *M* KCl, it is convenient to report an equivalent soil-water pH since it is a value that has more familiarity in relation to plant growth. The pH in 0.01 *M* $CaCl_2$ is approximately 0.6 units less than soil-water pH. The pH in 1 *M* KCl is approximately 0.9 units less than soil-water pH. A comparison of 1186 soils in Georgia provided an equation to calculate an equivalent soil-water pH from 0.01 *M* $CaCl_2$ soil pH as shown below.

   1:1 soil/water pH = 0.92 × (1:1 soil/0.01 *M* $CaCl_2$ soil pH) + 1.10 $r^2 = 0.91$

   A comparison of 240 soils in Kentucky provided an equation to calculate soil-water pH from 1 *M* KCl soil pH as shown below.

   1:1 soil/water pH = 0.91 × (1:1 soil/1 *M* KCl soil pH) + 1.34 $r^2 = 0.98$

2. Increased dilution of a soil/water mixture causes an increase in soil pH (Sikora, 2012). The pH in a 1:2 soil/water mixture is about 0.1 pH units greater than pH in a 1:1 soil/water mixture. A comparison of median values for 1:1 soil/water versus 1:2 soil/water from 134 samples in the North American Proficiency Testing program resulted in the following relationship.

   1:1 soil/water pH = 0.99 × 1:2 soil/water pH – 0.04 $r^2 = 1.00$

3. To determine if lime is needed, the measured pH is evaluated to determine if it is below some optimum pH for the specific crop to be grown (Kidder et al., 1988). Lime is then recommended to reach the desired soil-water pH which is some value between 5.5 to 7.0 for a 1:1 soil/water ratio. If using pH in 0.01 *M* $CaCl_2$ or 1 *M* KCl, correct the desired pH value to reflect the lower pH obtained in the electrolyte solution compared to pH measured in water.
4. To determine how much lime is needed to reach a target pH, refer to Chapters 3.3 through 3.8. These chapters present various methods to quantify lime requirement using buffers or $Ca(OH)_2$.

## Analytical Performance

Range and Sensitivity

1. Soil-water pH with a 1:1 soil/water ratio is most often within a range from 4.0 to 8.0 for soils in the Southeastern US.
2. Measurements of pH can be made to the nearest 0.1 or 0.01 pH unit. Measurements should not be made to more than 2 decimal places since this level of sensitivity is not achievable or required for lime requirement. If measurements are made to the nearest 0.01 pH unit, pH can be rounded to 0.1 pH units before reporting values to clients.

Precision and Accuracy

1. Examples of intralaboratory precision for pH in 1:1 soil/water, 1:1 soil/0.01 *M* $CaCl_2$, and 1:1 soil/1 *M* KCl are shown in Table 2.

*Table 2. Examples of intralaboratory variations from repeated soil pH measurements on separate samples for each method. Measurements were taken on different days using a LabFit robotic pH instrument.*

| Method | Number of measurements | pH Mean | pH standard deviation |
|---|---|---|---|
| 1:1 soil/water | 10 | 5.73 | 0.09 |
| 1:1 soil/0.01 *M* $CaCl_2$ | 10 | 5.47 | 0.04 |
| 1:1 soil/1 *M* KCl | 10 | 5.21 | 0.03 |

Interferences

1. Differences in pH will occur with the electrode placed in a soil-slurry versus the supernatant after the soil has settled. The differences are more pronounced with soil pH in water compared to electrolyte solutions. To avoid this variability in pH, it is important to stir the soil slurry right before measurement. With sandy soils, the settling time of soil particles is rapid and continuous stirring during measurement is recommended.
2. Glass electrodes have a short life span when measuring pH of sandy soils. The sand particles are abrasive to the glass resulting in electrode breakage or malfunction. Replace electrodes when pH of calibration buffers consistently exceed ± 0.05 pH units, even after recalibration, or when pH of quality control samples consistently exceed expected error.

## Interpretation

1. There is a wide variation of pH values for optimum plant growth. Most agronomic crops require soil-water pH values between 5.5 and 7. Some plants, such as blueberries and azaleas, require acidic soil conditions with soil-water pH below 5. Kidder et al. (1988) provide details on optimum pH ranges for various crops. A soil pH measurement provides an indication of whether lime is needed or not. If soil pH is below a certain value, some method of quantifying residual acidity is required to determine how much lime is needed to reach the desired pH. Chapters 3.3 through 3.8 provide various methods for quantifying soil acidity to determine lime requirement.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil pH measurement provided they are stored in an ammonia free environment or in a tightly sealed container.
2. Any pH meter, electrodes, or robotic instrument should be maintained and stored according to manufacturer instructions.

## Safety and disposal

1. The chemicals used in this procedure pose no safety risk and therefore can be stored and disposed of according to routine laboratory procedures.

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*Chapter 3.3*

# Single Addition of $Ca(OH)_2$ for Lime Requirement

*D.E. Kissel and L. Sonon*

## Application and Principle

The Single Addition $Ca(OH)_2$ method was designed to estimate a soil's pH buffering capacity, from which its lime requirement (LR) can be calculated. Kissel et al. (2007) developed the method to replace the use of the Adams-Evans buffer method to determine lime requirement for soils in Georgia. The method is based on the difference in the soil's initial pH and the pH measured 30 min after the addition of an aliquot of $Ca(OH)_2$. The difference in pH is used to calculate the pH buffering capacity of soil at a 30-min equilibration time. The method requires that titration curves of acid soils be linear in the working range from pH 4 to 6.5, which is the case for surface soils. The method was developed to work best with a robotic pH analyzer, although it could also be performed with manual pH meter measurements. Distinct advantages of the method include minimal amount of chemicals required and the resultant products after analysis being soil, water, and a small amount of $CaCl_2$ that can be safely disposed.

## Equipment and Apparatus

1. Soil scoop with 20 $cm^3$ capacity if measuring soil by volume
2. Analytical balance with 0.1 g resolution if measuring soil by weight
3. Analytical balance with 0.1 g resolution for making calcium hydroxide reagent
4. Analytical balance with 0.0001 g resolution for weighing potassium hydrogen phthalate to standardize calcium hydroxide
5. Burette for standardizing calcium hydroxide
6. Polyethylene carboy with 20-L capacity.
7. Glass, plastic, or waxed-lined paper cups with 50 mL capacity to hold soil and solution for pH measurements
8. Holding rack for cups
9. Volume dispensers for adding 20 mL 0.01 *M* $CaCl_2$ to soil
10. Volume dispenser for adding 2.7 mL saturated $Ca(OH)_2$ to soil
11. Manual pH meter or robotic pH analyzer
12. Glass pH electrode with an internal reference element or a separate reference electrode
13. Reciprocating shaker capable of 180 rpm

## Reagents

1. *Potassium hydrogen phthalate ($KHC_8H_4O_4$), 0.05 M KHP*: Crush 15 to 20 g of $KHC_8H_4O_4$ (formula weight = 204.23 g $m^{-1}$) to about 100 mesh and dry at 120$^oC$ for 2 h. Cool in a desiccator. Weigh 10.000 ± 0.001 g, transfer to a 1-L volumetric flask, and bring volume to 1 L with deionized water.

2. *Saturated calcium hydroxide ($Ca(OH)_2$)*: Weigh about 42 g $Ca(OH)_2$ powder and place it in a carboy container calibrated with a 20-L mark. Add approximately 10 L of deionized water and shake or stir vigorously to maximize dissolution of $Ca(OH)_2$. Fill the carboy to the 20-L mark. Cap tightly to prevent $CO_2$ from reacting with the $Ca(OH)_2$ solution. Shake or stir the solution again. Some $Ca(OH)_2$ particles remain undissolved and settle at the bottom of the container. The approximate concentration of $Ca(OH)_2$ in a saturated solution is 0.02 *M*.
   *Standardization of $Ca(OH)_2$:*
   a. Place 5 mL of 0.05 *M* KHP solution into a 100-mL beaker.
   b. Add 5 drops of 1% phenolphthalein indicator.
   c. Fill a burette with saturated $Ca(OH)_2$ solution.
   d. Slowly titrate saturated ($Ca(OH)_2$) into the KHP solution. When $OH^-$ from $Ca(OH)_2$ has neutralized hydrogen from KHP, the pink color of phenolphthalein appears. Stop the addition of calcium hydroxide when only the faintest pink appears and remains for 2 min. Record the volume of $Ca(OH)_2$ used.
   e. Calculate the molarity of $Ca(OH)_2$ as shown below.

      Molarity of $Ca(OH)_2$ = (5 mL × 0.05) ÷ (mL of $Ca(OH)_2$ used × 2)
      where 5 mL = volume of KHP
      0.05 = molarity of KHP
      2 = moles of KHP to react with one mole of $Ca(OH)_2$

3. *Phenolphthalein Indicator (1%)*: Available from chemical supply companies.

## Procedure

1. Measure 20 g or 20 $cm^3$ of processed soil (dried, < 2 mm) into a 50-mL sample cup.
2. Follow the procedure for making a 1:1 soil/0.01 *M* $CaCl_2$ pH measurement (see Chap 3.2).
3. Add 2.7 mL of saturated $Ca(OH)_2$ to the soil slurry to raise soil pH. The volume of $Ca(OH)_2$ added should be enough to give an increase in pH of at least 0.3 pH units.
4. Shake the soil $Ca(OH)_2$ mixture on an end to end shaker for 5 min and then let stand an additional 25 min before measuring pH. If using an automated pH analyzer, vigorously stir the soil and $Ca(OH)_2$ mixture and allow the suspension to equilibrate for 30 min before taking the soil pH measurement.
5. Place electrode in the soil slurry to measure pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for robotic instruments (see Chapter 3.2).

## Analytical Performance

Range and Sensitivity

1. The single addition titration can be used on very low buffered sandy soils to highly buffered fine-textured soils with high organic matter. For soils that vary widely in their lime buffer capacity (LBC), the addition of 2.7 mL of 0.023 *M* $Ca(OH)_2$ to 20 g of soil provides an adequate sensitivity for soils. With 2.7 mL $Ca(OH)_2$, a highly buffered soil with an LBC of

1000 mg $CaCO_3$ $kg^{-1}pH^{-1}$ would have a difference of 0.31 pH units between initial pH and pH taken 30 min after $Ca(OH)_2$ addition. Less buffered soils would have a larger difference in pH; for example, an LBC of 500 mg $CaCO_3$ $kg^{-1}$ $pH^{-1}$ would have a pH difference of 0.62 pH units.

2. The pH measurements can be made to the nearest 0.01 pH unit. Calculation of the LBC should be based on pH values to the nearest 0.01 pH unit. The greater accuracy in pH is needed to maximize accuracy of LBC which is calculated from the initial pH minus the pH taken after $Ca(OH)_2$ addition. It is also advisable to take the initial pH and second pH with the same pH electrode.

Precision and Accuracy

1. Typical measurement of intralaboratory precision for LBC is shown in Table 1.

*Table 1. Example intralaboratory variation of LBC determined on a LabFit robotic pH instrument for one soil with 10 consecutive measurements using the same electrode.*

| Method | Number of measurements | LBC Mean | LBC Standard deviation |
|---|---|---|---|
| | | ---- (mg $CaCO_3$ $(kg\ soil)^{-1}$ $pH^{-1}$) ---- | |
| Single Addition $Ca(OH)_2$ | 10 | 189 | 9.1 |

Interferences

1. There are no known interferences with the $Ca(OH)_2$ titration method.
2. The electrode should have a protective sleeve when measuring pH in sandy soils to protect the glass pH bulb from abrasive sand particles. Electrode life is lengthened with the protective sleeve.

## Calculations and Interpretation

1. Calculation of lime requirement (LR) requires the determination of the soil's pH buffer capacity. For convenience and better understanding by clients, LR is expressed in units of $CaCO_3$ and is called lime buffer capacity (LBC). The LBC is calculated with the following equation

$$LBC_{30min} = (V \times M \times 100.1\ g\ mole^{-1}) \times (0.02\ kg\ soil)^{-1} \div (pH_2 - pH_1) \quad [1]$$

where $LBC_{30min}$ has units of mg $CaCO_3$ $(kg\ soil)^{-1}$ $pH^{-1}$, V is the volume (mL) of $Ca(OH)_2$ added, *M* is the molarity of the saturated $Ca(OH)_2$, 100.1 g $mole^{-1}$ is the molecular weight of $CaCO_3$, $pH_2$ is soil pH 30 min after adding $Ca(OH)_2$, and $pH_1$ is the initial soil pH. The mass of soil is shown as 0.02 kg. The actual mass may be slightly greater than 0.02 kg if soil was measured by volume with a 20 $cm^3$ scoop. The equation assumes that $CO_3^{2-}$ anions react

identically to a chemically equivalent amount of $OH^-$ ions (Kissel et al., 1988 and Liu et al., 2008).

2. The LR, expressed as reagent-grade powdered $CaCO_3$, is calculated from the following equation developed by Kissel et al. (2007).

   $$LR\ (mg\ kg^{-1}) = LBC_{30min} \times (target\ pH_w - pH_{CaCl2}) \quad [2]$$

   where $LBC_{30min}$ is the soil's LBC from Eq. [1] after 30 min equilibration with $Ca(OH)_2$, target $pH_w$ is the target pH in water, and $pH_{CaCl2}$ is the pH measured in 0.01 *M* $CaCl_2$ before the addition of $Ca(OH)_2$. The target $pH_w$ is used rather than target $pH_{CaCl2}$ because, as noted by Liu et al. (2005), the 30-min equilibration time for the $Ca(OH)_2$ is not sufficient to reach a final equilibrium pH with the soil acidity. Since the target $pH_w$ is numerically greater than a target $pH_{CaCl2}$, the value of (target $pH_w$ – $pH_{CaCl2}$) is larger and makes up for a smaller value of LBC due to the lack of complete equilibrium. Based on the data presented by Kissel et al. (2009), the average difference in $pH_w$ and $pH_{CaCl2}$ for approximately 1200 soil samples was 0.6 units. Because $pH_w$ is approximately 0.6 units greater than $pH_{CaCl2}$, Eq [2] can be rearranged to

   $$LR\ (mg\ kg^{-1}) = LBC_{30min} \times [(target\ pH_{CaCl2} + 0.6) - pH_{CaCl2}] \quad [3]$$

3. An alternative and improvement to the use of Eq. [3] for calculating LR is to determine the LBC for which a soil is at pH equilibrium ($LBC_{Eq}$) after adding $Ca(OH)_2$. Studies were done for a wide range of Georgia soils to obtain a calibration equation for $LBC_{Eq}$ as a function of $LBC_{30min}$. Thompson et al. (2010) first showed that pH equilibrium after adding $Ca(OH)_2$ was achieved within five days for a wide range of Georgia soils. He also found that $LBC_{Eq}$ could be predicted from $LBC_{30min}$ determined in the laboratory. Further work by Kissel et al. (2012) included more soils, and showed that $LBC_{Eq}$ determined with a 5 day incubation could be predicted as a linear function of $LBC_{30min}$. The final working equations now used by the University of Georgia (Sonon and Kissel, 2012) are:

   a. Soils with $LBC_{30min} < 250$: $LBC_{Eq} = (3.67 \times LBC_{30min}) - 188.3$
   b. Soils with $LBC_{30min} \geq 250$: $LBC_{Eq} = 2.90 \times LBC_{30min}$

   Once the $LBC_{Eq}$ has been calculated, the LR equation then becomes

   $$LR\ (mg\ kg^{-1}) = LBC_{Eq} \times (target\ pH_{CaCl2} - pH_{CaCl2}) \quad [4]$$

4. Equations [2] and [4] determine LR as reagent grade $CaCO_3$ per kg soil. With a sampling depth of 6 inches and assumed soil weight of 2 million pounds per acre, the following can be used to determine LR as tons ag lime $acre^{-1}$.

   $$LR\ (tons\ ag\ lime\ acre^{-1}) = LR\ (mg\ kg^{-1}) \times 0.001 \times (100 \div \%\ lime\ effectiveness)$$

   The 0.001 factor converts mg $kg^{-1}$ of reagent grade $CaCO_3$ to tons $acre^{-1}$ reagent grade $CaCO_3$. The factor of (100 ÷ % lime effectiveness) converts the amount of reagent grade

$CaCO_3$ to the amount of ag lime where % lime effectiveness is determined by calcium carbonate equivalence and particle size. Common lime effectiveness for ag lime is 67% which makes this factor 1.5.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting pH measurements provided they are stored in an ammonia-free environment or in a tightly sealed container.
2. The apparatus used for determining the pH should be maintained and stored according to the manufacturer instructions.
3. The $Ca(OH)_2$ solution has an undetermined but long shelf life due to its high pH greater than 12. It must be protected from $CO_2$ with an ascarite trap to scrub $CO_2$ from air that enters the carboy as solution is withdrawn.

## Safety and Disposal

1. The chemicals used in this procedure pose no safety risk and therefore can be stored and disposed of according to routine laboratory procedures.
2. After reaction of $Ca(OH)_2$ with soil, only soil and a dilute concentration of $CaCl_2$ remain with pH levels between 4 and 10. This mixture can be disposed of without special precautions according to routine laboratory procedures.

## References


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*Chapter 3.4*

# Sikora Buffer for Lime Requirement

*F.J. Sikora*

## Application and Principle

The Sikora buffer method (Sikora, 2006) was designed to reproduce the same soil-buffer pH obtained with the SMP buffer method, but without the hazardous chemicals contained in the SMP buffer. The SMP buffer was developed by Shoemaker, McLean and Pratt (Shoemaker et al., 1961) to obtain lime requirement for soils in Ohio which are silt loams that can contain appreciable extractable Al. Two of the bases in the SMP buffer, p-nitrophenol and chromate, are considered hazardous and thus require special protocols be followed for disposal (USEPA 1980a, USEPA 1980b). The Sikora buffer was developed to reproduce the same soil-buffer pH obtained with SMP buffer by replacing the hazardous chemicals with imidazole and 2-(N-morpholino) ethanesulfonic acid monohydrate (MES). Because of its identical pH response to added $H^+$, the Sikora buffer is suited for the same type of soils where SMP buffer is used. Using Sikora buffer to replace SMP buffer removes the need to handle hazardous waste and requires no change in interpreting data for lime recommendations.

## Equipment and Apparatus

1. Soil scoop with 10 $cm^3$ volume if measuring soil by volume
2. Analytical balance with 0.1 g resolution if measuring soil by weight
3. Analytical balance with 0.01 g resolution for making Sikora buffer
4. Glass, plastic, or waxed-lined paper cups with 50 mL capacity to hold soil and solution for pH measurements
5. Holding rack for sample cups
6. Volume dispensers for adding 10 mL deionized water and 10 mL Sikora buffer to soil
7. Manual pH meter or robotic pH analyzer
8. Glass pH electrode with an internal reference element or a separate reference electrode
9. Reciprocating shaker capable of 180 rpm

## Reagents

1. *Sikora buffer*: Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The buffer contains 2 *M* potassium chloride, 0.089 *M* acetic acid, 0.031 *M* MES, 0.014 *M* imidazole, and 0.070 *M* triethanolamine with a pH of 7.70.
   a. Add 149 g of potassium chloride (KCl) in about 750 mL of deionized water in a 1 L volumetric flask and stir thoroughly to dissolve.
   b. Add 5.11 mL, or 5.36 g, of glacial acetic acid ($CH_3COOH$) and stir.

c. Add 6.70 g of 2-(N-Morpholino)ethanesulfonic acid monohydrate (MES; $C_6H_{13}NO_4S{\cdot}H_2O$) and stir to dissolve.
d. Add 0.936 g of imidazole ($C_3H_4N_2$) and stir to dissolve.
e. Add 9.23 mL, or 10.38 g, of triethanolamine (TEA; $(HOCH_2CH_2)_3N$) and stir to dissolve. For ease of delivery, 18.5 mL of 50% (v/v) TEA can be added.
f. Add 5 mL of 40% (w/w) sodium hydroxide (NaOH) and stir.
g. Bring solution volume to 1 L with deionized water.
h. Adjust the pH of the solution to 7.70 with dropwise addition of 40% (w/w) NaOH or 50% (v/v) HCl.

*pH verification:*

i. Mix 50 mL of Sikora buffer with 50 mL of deionized water. The pH of the solution should be 7.53±0.03.
j. Add 5 mL of 0.5 *M* HCl to the 1:1 mixture of Sikora buffer and deionized water. The pH of the solution should be 5.68±0.07.

## Procedure

1. Measure 10 g or 10 $cm^3$ of processed soil (dried, < 2 mm) into a sample cup. The volume measurement adds approximately 11.8 g with an assumed soil density of 1.18 g $cm^{-3}$. Soil can alternatively be measured with an 8.5 $cm^3$ scoop that would measure approximately 10 g with assumed density of 1.18 g $cm^{-3}$.
2. Follow the procedure for making a 1:1 soil/water pH measurement (see Chapter 3.2).
3. Add 10 mL of Sikora buffer to the soil slurry.
4. Shake the soil/water/buffer solution on an end-to-end shaker for 10 min and let set for 30 min after shaking. Alternatively, shake for 15 min and let set for 15 min after shaking. If using a robotic pH analyzer, stir the buffer with soil vigorously and allow solution to set for at least 30 min.
5. Place electrode in the soil slurry to measure soil-buffer pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for robotic pH instruments (see Chapter 3.2).

## Analytical Performance

Range and Sensitivity

1. Soil-buffer pH is most often within a range of 6.3 to 7.5. For very acidic soils, soil-buffer pH can be as low as 5.3. The Sikora buffer was designed to reproduce SMP soil-buffer pH values from 5.3 to 7.5.
2. Soil-buffer pH measurements can be made to the nearest 0.1 or 0.01 pH unit. If measurements are made to the nearest 0.01 pH unit, pH can be rounded to 0.1 pH units before reporting to clients.

Precision and Accuracy

1. Typical measurements of intralaboratory precision for Sikora soil-buffer pH are shown in Table 1.

*Table 1. Example intralaboratory variations for soil-buffer pH using the Sikora buffer on four different soils. Measurements were taken on different days using a LabFit robotic pH instrument.*

| Soil texture | Number of measurements | Soil-buffer pH Mean | Soil-buffer pH Standard deviation |
|---|---|---|---|
| Silt loam | 10 | 6.54 | 0.05 |
| Silt loam | 10 | 7.06 | 0.06 |
| Loamy sand | 10 | 7.18 | 0.02 |
| Sandy loam | 10 | 6.88 | 0.04 |

Interferences

1. The Sikora soil-buffer pH reaches stability quicker than pH measured in deionized water due to pH buffering capacity and electrolytes of the buffer. Differences in pH may occur with the electrode placed in a soil-slurry compared to the supernatant after soil has settled. To avoid this variability in pH, it is important to stir the soil slurry during or prior to measurement.
2. Rinsing the electrode in between samples is not as important with soil-buffer pH measurements compared to soil/water pH or soil-salt pH measurements (see Chapter 3.2). The effect of soil carryover from one sample to the next is minimal due to the pH buffer capacity of the buffer.
3. The junction potential of electrodes can become clogged during storage or after repeated measurements. Make sure to clean electrodes periodically to ensure proper measurements. When electrodes fail to measure pH of calibration buffers or quality control samples show more error than expected, replace electrodes.

## Interpretation

1. Since the Sikora buffer was designed to reproduce soil-buffer pH obtained with the SMP buffer, the reader is referred to Chapter 3.7 on the SMP buffer method for interpreting data for lime requirement. Many laboratories have different recommendation tables based on calibration with soils in their region. The Sikora buffer can replace the SMP buffer using lime recommendation tables developed for the SMP buffer method since the same soil-buffer pH value is obtained (Sikora, 2006).

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil-buffer pH measurement provided they are stored in an ammonia free environment or in a tightly sealed container.
2. Any pH meter, electrodes, or robotic instrument should be maintained and stored according to manufacturer instructions.
3. The Sikora buffer was designed to have a long shelf-life. Research has shown that buffer stored for 90 (Nathan et al., 2012) or 150 (Sikora, 2006) days showed no observable microbial growth and no change in soil/buffer pH measurements. During routine use of the

buffer in soil testing, a black microbial growth does occur on interior walls of plastic storage containers. The microbial growth is more prominent when the solution is exposed to air. The microbial growth also appears in the tubing and dispensers of robotic pH instruments. The microbial growth does not affect the soil-buffer pH measurements but should be controlled to keep containers and delivery tubes in robotic pH instruments clean. The microbial growth in storage containers of the buffer can be controlled by thorough soaking and cleaning of the containers with mild bleach (10% v/v) with adequate rinsing and drying time before adding buffer for storage. Keeping the buffer stored in a closed container reduces growth. Tubing of automated pH instruments should also be rinsed periodically with mild bleach (10% v/v).

## Safety and disposal

1. The chemicals used in this procedure do not require disposal according to hazardous waste protocols. Handle all chemicals according to routine laboratory procedures.

## References


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*Chapter 3.5*

# Sikora-2 Buffer for Lime Requirement

*F.J. Sikora*

## Application and Principle

The Sikora-2 buffer is used to determine soil-buffer pH after soil pH is measured in 1 *M* KCl ($pH_{KCl}$). Soil-buffer pH and $pH_{KCl}$ are used to construct a lime response curve for determining lime requirement. The Sikora-2 buffer method has advantages of accounting for the pH buffering capacity of each soil tested and removing seasonal soil pH variability.

Soil pH is most often obtained in a 1:1 mixture of soil and deionized water. If salt is present in soil, soil pH in water can be 0.2 to 1 pH units lower than when measured in the same soil with little to no salt. Cations from the free salt can either affect liquid junction potential or exchange with $Al^{3+}$ and $H^{+}$ on soil colloids to result in lower pH. Salt can increase during the season due to fertilizer application, organic matter mineralization, and drought conditions. Salt can also be at very low levels following heavy rains. To avoid variable soil pH measured with water, soil pH measurements can be made with electrolyte solutions such as 0.01 *M* $CaCl_2$ or 1 *M* KCl (see chapter 3.2).

The Sikora-2 buffer was developed to be used following measurement of $pH_{KCl}$. This is in contrast to use of Sikora buffer used after measurement of soil-water pH. The soil-buffer pH obtained with Sikora-2 buffer is used in conjunction with $pH_{KCl}$ to construct a two-point lime response curve from which a lime requirement is determined. This method provides the same benefit obtained with double buffer methods (McLean et al., 1977; Yuan, 1974; Sikora, 2012) and the Single Addition $CaOH_2$ method (Kissel et al., 2007; Liu et al., 2005; Chapter 3.3) where the pH buffer capacity of soil is determined for each soil tested.

## Equipment and Apparatus

1. Soil scoop with 10 $cm^3$ volume if measuring soil by volume
2. Analytical balance with 0.1 g resolution if measuring soil by weight
3. Analytical balance with 0.01 g resolution for making Sikora-2 buffer
4. Glass, plastic, or waxed-lined paper cups with 50 mL capacity to hold soil and solution for pH measurements
5. Holding rack for sample cups
6. Volume dispensers for adding 10 mL deionized water and 10 mL Sikora-2 buffer to soil
7. Manual pH meter or robotic pH analyzer
8. Glass pH electrode with an internal reference element or a separate reference electrode
9. Reciprocating shaker capable of 180 rpm

## Reagents

1. *Sikora-2 buffer*: The differences between Sikora-2 buffer and Sikora buffer (Chapter 3.5) are that half the amount of KCl is used and the pH of the buffer is adjusted to 7.53 rather than 7.70. Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The buffer contains 1 *M* potassium chloride, 0.089 *M* acetic acid, 0.031 *M* MES, 0.014 *M* imidazole, and 0.070 *M* triethanolamine with a pH of 7.53.
   a. Add 74.5 g of potassium chloride (KCl) in about 750 mL of deionized water in a 1 L volumetric flask and stir thoroughly to dissolve.
   b. Add 5.11 mL g, or 5.36 g, of glacial acetic acid ($CH_3COOH$) and stir.
   c. Add 6.70 g of 2-(N-Morpholino)ethanesulfonic acid monohydrate (MES; $C_6H_{13}NO_4S{\cdot}H_2O$) and stir.
   d. Add 0.936 g of imidazole ($C_3H_4N_2$) and stir.
   e. Add 9.23 mL, or 10.38 g, of triethanolamine (TEA; $(HOCH_2CH_2)_3N$) and stir. For ease of delivery, 18.5 mL of 50% (v/v) TEA can be added.
   f. Add 5 mL of 40% (w/w) sodium hydroxide (NaOH) and stir.
   g. Bring solution volume to 1 L with deionized water.
   h. Adjust the pH of the solution to 7.53 with dropwise addition of 40% (w/w) NaOH or 50% (v/v) HCl.

   *pH verification:*
   i. Mix 50 mL of Sikora-2 buffer with 50 mL of 1 M KCl. The pH of the solution should be 7.53±0.03.
   j. Add 5 mL of 0.5 *M* HCl to the 1:1 mixture of Sikora-2 buffer and 1 M KCl. The pH of the solution should be 5.68±0.07.

## Procedure

1. Measure 10 g or 10 $cm^3$ of processed soil (dried, < 2 mm) into a sample cup. The volume measurement is approximately 11.8 g with an assumed soil density of 1.18 g $cm^{-3}$. Soil can alternatively be measured with an 8.5 $cm^3$ scoop that would measure approximately 10 g with assumed density of 1.18 g $cm^{-3}$.
2. Follow the procedure for determining $pH_{KCl}$ in 1:1 soil/1 *M* KCl (see Chapter 3.2).
3. Add 10 mL of Sikora-2 buffer to the soil slurry.
4. Shake the soil/water/buffer solution on an end-to-end shaker for 10 min and let set for 30 min after shaking. Alternatively, shake for 15 min and let set for 15 min after shaking. If using an automated pH analyzer, stir the buffer with soil vigorously and allow solution to set for at least 30 min.
5. Place electrode in the soil slurry to measure soil-buffer pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for robotic pH instruments (see Chapter 3.2).

## Analytical Performance

Range and Sensitivity

1. Soil-buffer pH is most often within a range of 6.3 to 7.5. For very acidic soils, the soil-buffer pH can be as low as 5.3. The Sikora-2 buffer was designed to reproduce SMP soil-buffer pH values from 5.3 to 7.5.
2. Soil-buffer pH measurements can be made to the nearest 0.1 or 0.01 pH unit. If measurements are made to the nearest 0.01 pH unit, pH can be rounded to 0.1 pH units before reporting to clients.

Precision and Accuracy

1. Typical measurements of intralaboratory precision for Sikora-2 soil-buffer pH are shown in Table 1.

*Table 1. Example intralaboratory variations for soil-buffer pH using the Sikora-2 buffer on four different soils. Measurements were taken on different days using a LabFit robotic pH instrument.*

| Soil texture | Number of measurements | Soil-buffer pH Mean | Soil-buffer pH Standard deviation |
|---|---|---|---|
| Silt loam | 10 | 6.42 | 0.05 |
| Silt loam | 10 | 6.96 | 0.05 |
| Loamy sand | 10 | 7.24 | 0.02 |
| Sandy loam | 10 | 6.96 | 0.03 |

Interferences

1. The soil-buffer pH reaches stability quicker than pH measured in deionized water due to pH buffering capacity and electrolytes of the buffer. Differences in pH may occur with the electrode placed in a soil-slurry compared to the supernatant after soil has settled. To avoid this variability in pH, it is important to stir the soil slurry during or prior to measurement.
2. Rinsing the electrode in between samples is not as important with soil-buffer pH measurements compared to soil-water pH or soil-salt pH measurements (see Chapter 3.2). The effect of soil carryover from one sample to the next is minimal due to the pH buffer capacity of the buffer.
3. The junction potential of electrodes can become clogged during storage or after repeated measurements. Make sure to clean electrodes periodically to ensure proper measurements. When electrodes consistently fail to measure pH of calibration buffers or quality control samples consistently show more error than expected, replace electrodes.

## Calculations and Interpretation

1. The Sikora-2 soil-buffer pH and $pH_{KCl}$ provide a lime response curve from which a lime requirement can be determined (Fig. 1). A lime response curve can be generated from these two pH values because both pH measurements are made in 1 *M* KCl. Lime requirement is then calculated as:

   Lime requirement = Alkalinity (cmol $kg^{-1}$) to reach target $pH_{KCl}$ =
   (target $pH_{KCl}$ – $pH_{KCl}$) × (soil-buffer pH – 7.55) ÷ [ (soil-buffer pH – $pH_{KCl}$) × (-0.364) ]
   × 10 ÷ (g soil) [1]

   The target $pH_{KCl}$ from the example in Fig. 1 is 5.56 which corresponds to a soil-water pH ($pH_w$) of 6.4 according to the relationship between $pH_{KCl}$ and $pH_w$ (see chapter 3.2). Any target $pH_{KCl}$, corresponding to a desired $pH_w$, can be used in Eq. [1]. Derivation of Eq. [1] is provided in the Appendix.

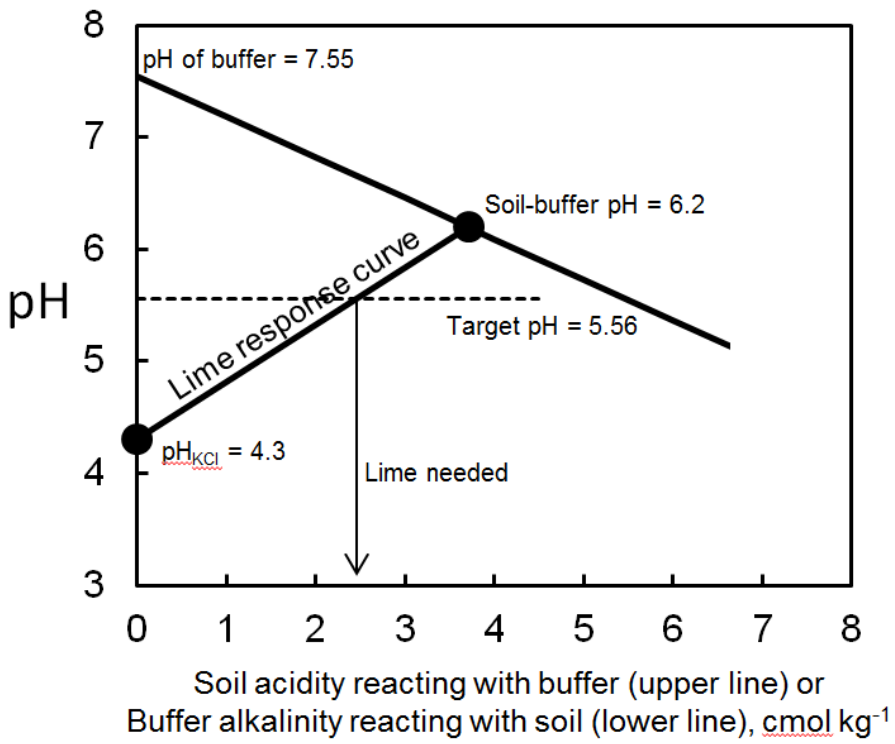


*Fig. 1. An example lime-response curve with $pH_{KCl}$ of 4.3 and Sikora-2 soil-buffer pH of 6.2. 2.46 cmol $kg^{-1}$ alkalinity (equivalent to 1.23 tons $acre^{-1}$ $CaCO_3$) is required to increase pH to a target $pH_{KCl}$ of 5.56 which is equivalent to $pH_w$ of 6.4.*

Lime requirement determined from Eq. [1] underestimates lime needed for field application because pH measurements are made with short-term reactions of alkali and soil after only 30 min. Soil acidity reacts slowly with lime. Also, lime applied in the field may not be mixed

thoroughly with soil as occurs in the soil-buffer pH determination. Therefore, a correction factor must be multiplied by lime requirement determined from Eq. [1] to obtain a proper lime requirement for field application.

Prior to 2005, University of Kentucky lime recommendations were based on a combination of soil-water pH and SMP soil-buffer pH (University of Kentucky, 2010). The recommendations were validated with $Ca(OH)_2$ titrations of 33 Kentucky soils (Dollarhide et al., 1995). In July 2005, the Sikora buffer was used to replace the SMP buffer which gave the same soil-buffer pH (see Chapter 3.4). The Sikora-2 buffer described in this method gives the same soil-buffer pH as SMP and Sikora buffer. The only difference is that Sikora-2 is designed to be added to a mixture of soil and 1 M KCl while the Sikora buffer was designed to be added to a mixture of soil and water. Benefits derived from using 1 M KCl in the first pH measurement include removing seasonal variation in soil pH measurements and providing a means of generating a lime response curve for each soil tested.

The University of Kentucky lime recommendations were used to obtain multiplicative factors for correcting lime requirement from the Sikora-2 buffer measurement (Eq. [1]) to lime requirement for field application. The correction factors for lime were observed to depend on the lime requirement from Eq. [1] as shown below.

With Lime Requirement from Eq. [1] $\leq$ 6 cmol $kg^{-1}$ :
Correction factor = 3.62 – (0.367 × (Lime Requirement from Eq. [1]) ) [2]

With Lime Requirement from Eq. [1] > 6 cmol $kg^{-1}$ :
Correction factor = 1.42 [3]

Lime requirement is first determined from $pH_{KCl}$ and Sikora-2 soil-buffer pH using Eq. [1]. This value is then multiplied by a correction factor determined from Eq. [2] or [3]. The result of the calculation is in units of cmol $kg^{-1}$ $CaCO_3$. This value is divided by two to obtain tons $acre^{-1}$ $CaCO_3$ and can be rounded to the nearest 0.25 tons $acre^{-1}$. The resultant lime rate is for pure $CaCO_3$ at a six inch soil depth. Further calculation is required to account for the quality of agricultural limestone and for incorporating lime to different soil depths.

2. Equation [1] can be modified to determine lime requirement with $pH_w$ and soil-buffer pH determination using Sikora or SMP buffers. Both Sikora and SMP buffer methods were designed to have buffer added after measurement of $pH_w$. Soil-buffer pH values from Sikora or SMP buffers are equivalent to soil-buffer pH from Sikora-2 buffer so either of the values can be entered directly into Eq. [1]. Soil-water pH can be introduced into Eq. [1] from the relationship between $pH_{KCl}$ and $pH_w$ observed for 240 Kentucky soils as shown below.

$$pH_{KCl} = 1.10 \times (pH_w) - 1.47 \quad [4]$$

Substituting $pH_{KCl}$ from Eq. [4] into Eq. [1] and rearranging results in the following equation.

Lime requirement = Alkalinity (cmol $kg^{-1}$) to reach target $pH_w$ =
$-1.10 \times (\text{target } pH_w - pH_w) \times (\text{soil-buffer pH} - 7.55) \div [\ \text{soil-buffer pH} - (1.10 \times pH_w) + 1.47\ ] \times 27.5 \div (\text{g soil})$ [5]

Entering target $pH_w$, measured $pH_w$, and soil-buffer pH from Sikora or SMP buffer provides lime requirement in cmol $kg^{-1}$ to reach the target $pH_w$. The value is multiplied by a correction factor from Eqs. [2] or [3], using lime requirement from Eq. [5], and then divided by 2 to obtain a lime requirement for field application in tons $acre^{-1}$ of pure $CaCO_3$ at a six inch soil depth. Further calculation is required to account for the quality of agricultural limestone and for incorporating lime to different soil depths.

3. To adjust to a rate of agricultural limestone that is less effective than pure $CaCO_3$, multiply the $CaCO_3$ rate by 100 / (% effectiveness of agricultural limestone). A common % effectiveness of agricultural limestone is 67% which results in a multiplication factor of 1.5.
4. To adjust lime requirement to a soil depth other than 6 inches, multiply the agricultural limestone rate by (desired soil depth in inches) / 6. For a soil depth of 8 inches, the multiplication factor would be 1.33.
5. This method has been used at the University of Kentucky since January, 2010. The main advantages of the method are 1) a salt pH is determined which removes the influence background salt has on soil-water pH and 2) pH buffer capacity is determined for each individual soil tested (Sikora, 2012).

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil-buffer pH measurement provided they are stored in an ammonia free environment or in a tightly sealed container.
2. Any pH meter, electrodes, or robotic instrument should be maintained and stored according to manufacturer instructions.
3. The Sikora-2 buffer is very similar to the Sikora buffer that was observed to be stored for 90 (Nathan et al., 2012) or 150 (Sikora, 2006) days with no observable microbial growth and change in soil-buffer pH measurements. During routine use of the buffer in soil testing, a black microbial growth does occur on interior walls of plastic storage containers. The microbial growth is more prominent when the solution is exposed to air. The microbial growth also appears in the tubing and dispensers of robotic pH instruments. The microbial growth does not affect the soil-buffer pH measurements but should be controlled to keep containers and delivery tubes in robotic pH instruments clean. The microbial growth in storage containers of the buffer can be controlled by thorough soaking and cleaning of the containers with mild bleach (10% v/v) with adequate rinsing and drying time before adding buffer for storage. Keeping the buffer stored in a closed container reduces growth. Tubing of automated pH instruments should also be rinsed periodically with mild bleach (10% v/v).

## Safety and disposal

1. The chemicals used in this procedure do not require disposal according to hazardous waste protocols. Handle all chemical according to routine laboratory procedures.

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## Appendix (Derivation of Eq. [1])

With 1:1 Sikora-2 buffer/water mixture titrated with HCl, the relationship between pH and acidity was observed to be described by a linear equation as shown below (Sikora, 2012).

$$pH = 7.55 - 0.364 \times (\text{acidity, cmol kg}^{-1}) \quad [6]$$

Acidity is shown in units of cmol $kg^{-1}$ assuming the acidity from HCl comes from a 1:1 weight to volume mixture of soil/water.

When buffer is added to soil, acidity from the soil reacts with the buffer to depress the initial pH of the buffer. Assume a condition with soil pH ($pH_{KCl}$) of 4.3 and a soil-buffer pH of 6.2 (Fig. 1). Using 6.2 for pH in Eq. [6] and solving for acidity reveals 3.71 cmol $kg^{-1}$ of soil acidity reacted with the buffer to depress pH of the buffer from 7.55 to 6.2. The quantity of soil acidity reacting with the buffer to depress the buffer pH is equivalent to the quantity of buffer alkalinity reacting with soil acidity to increase soil pH. Therefore, another accurate statement is that 3.71 cmol $kg^{-1}$ of buffer alkalinity reacted with soil acidity to increase $pH_{KCl}$ from an initial value of 4.3 to 6.2.

The $pH_{KCl}$ and the soil-buffer pH values provide two points on a lime response curve with pH versus applied alkalinity at an ionic strength of 1 M. The (x,y) coordinates of the two points can be represented as shown below.

(x,y) coordinate of 1st point at 0 alkalinity = $(0 , pH_{KCl})$ [7]

(x,y) coordinate of 2nd point at soil-buffer pH =
((soil-buffer pH – 7.55) ÷ (-0.364) , soil-buffer pH ) [8]

The x-coordinate of the second point is alkalinity required to increase soil pH to the soil-buffer pH and comes from algebraic manipulation of Eq. [6] since applied alkalinity reacting with soil acidity to increase soil pH is equivalent to soil acidity reacting with buffer alkalinity to decrease initial buffer pH.

Since the two points generate a line representing the lime response curve, the amount of alkalinity required to increase soil pH to any value can be calculated. The lime response curve represents pH at an ionic strength of 1 M. To determine alkalinity to raise pH to a target $pH_w$, a relationship between $pH_{KCl}$ and $pH_w$ must be established. The relationship observed for 240 Kentucky soils is shown below.

$$pH_{KCl} = 1.099 \times pH_w - 1.473 \quad [9]$$

A target $pH_w$ of 6.4 is equivalent to $pH_{KCl}$ of 5.56 according to Eq. [9]. To determine the amount of alkalinity to increase $pH_w$ to 6.4, a third point needing to be considered on the lime response curve is the point with $pH_{KCl}$ equal to 5.56 as the y coordinate. The (x,y) coordinate of this third point can be represented as shown below.

(x,y) coordinate of 3rd point at target pH value =
(alkalinity required to reach target pH , target $pH_{KCl} = 5.56$) [10]

The x coordinate of the third point is an unknown that needs to be calculated. The three points and linear algebra are used to determine this unknown. The slope of the lime response curve is derived from the first and second points as shown below.

Slope of lime response curve =
(y coordinate of 2nd point – y coordinate of 1st point) ÷ (x coordinate of 2nd point) =
(soil-buffer pH – $pH_{KCl}$) ÷ ((soil-buffer pH – 7.55) ÷ (-0.364))

The intercept of the lime response curve is $pH_{KCl}$.

The equation of the lime response curve including the target $pH_{KCl}$ (y) and alkalinity required to reach the target $pH_{KCl}$ (x) takes the form of the linear equation, y = slope × x + intercept, as shown below.

Target $pH_{KCl} = 5.56 =$

$$\frac{(\text{soil-buffer pH} - \text{pH}_{KCl})}{((\text{soil-buffer pH} - 7.55) \div (-0.364))} \times \text{alkalinity to reach target pH} + \text{pH}_{KCl} \quad [11]$$

Rearranging Eq. [11] to solve for alkalinity to reach target pH results in the equation below for a target $pH_{KCl}$ of 5.56 which is equivalent to $pH_w$ of 6.4.

$$\text{Alkalinity (cmol kg}^{-1}\text{) to reach target pH} = (5.56 - \text{pH}_{KCl}) \times (\text{soil-buffer pH} - 7.55) \div [\ (\text{soil-buffer pH} - \text{pH}_{KCl}) \times (-0.364)\ ] \quad [12]$$

Equation [12] is for a 1:1 weight to volume mixture of soil/water to which buffer was added. This equation would be used for a mixture of 10 g of soil, 10 mL of water, and 10 mL of Sikora-2 buffer. If a 10 $cm^3$ scoop is used to measure soil, soil weight will be a little more than 10 g. For silt loam soils with an average density of 1.18 g $cm^{-3}$, soil weight will be 11.8 g. To correct for a weight of soil that is different from 10 g, Eq. [12] is multiplied by 10 ÷ (g soil).

$$\text{Alkalinity (cmol kg}^{-1}\text{) to reach target soil-water pH of 6.4} = (5.56 - \text{pH}_{KCl}) \times (\text{soil-buffer pH} - 7.55) \div [\ (\text{soil-buffer pH} - \text{pH}_{KCl}) \times (-0.364)\ ] \times 10 \div (\text{g soil}) \quad [13]$$

*Chapter 3.6*

# Mehlich and Modified Mehlich Buffers for Lime Requirement

*D.H. Hardy*

## Application and Principle

The Mehlich buffer was developed to estimate acidity affecting plant growth in North Carolina's mineral (sandy coastal plains and clay piedmont soils) and organic (Histosols and mineral soils with Histic epipedons) soils in order to provide a lime requirement (LR). Earlier methods to measure acidity and estimate lime requirements for mineral soils were based on 1 *M* KCl extraction of exchangeable $Al^{+3}$ or soil solution $Al^{+3}$ (Evans and Kamprath, 1970; Kamprath, 1970). The Mehlich buffer method (Mehlich, 1976) was developed to replace these methods for routine soil testing since the $Al^{+3}$ extraction methods were too time-consuming.

The Mehlich buffer primarily measures exchangeable acidity ($H^+$ and $Al^{+3}$) through displacement of these acidic cations by $Ba^{2+}$ and $NH_4^+$. Subsequent appearance of $H^+$ in solution occurs through direct displacement from the cation exchange sites or through hydrolysis of the displaced exchangeable $Al^{+3}$. The underlying premise is that a decline in pH of the buffer is linearly related to the displaced exchangeable acidity. Since the pH is buffered at 6.6, some residual acidity is also measured.

In his published work (Mehlich, 1976), Mehlich's measurement of buffer acidity was related to exchangeable acidity measured with an unbuffered neutral salt ($BaCl_2$) and residual acidity using $BaCl_2$ and triethanolamine at pH 8.25. Mehlich specified no specific target pH in calculating lime requirements but proposed various lime equations based on organic matter content, mineral composition of soils, and plant tolerance to exchangeable acidity as derived from concomitant field and greenhouse studies. In 1984, Mehlich modified these equations into one equation currently used by North Carolina to make lime recommendations with different target pH values based primarily upon soil classes as mineral, mineral-organic, or organic.

In 2005, a modified Mehlich buffer was developed and is used by soil testing laboratories in Virginia, Pennsylvania, and Maine. The modified Mehlich buffer functions under the same principle as the original buffer, except barium chloride is replaced by calcium chloride in the buffer. This modification occurred to remove the protocols for hazardous waste disposal due to the presence of barium. Lime recommendations given by the states using modified Mehlich buffer are based upon calibration equations derived from lime incubation studies of their native soils (Hoskins and Erich, 2008; Wolf et al., 2008).

## Equipment and Apparatus

1. Soil scoop with 10 $cm^3$ volume
2. Analytical balance with 0.01 g resolution for making Mehlich or modified-Mehlich buffer
3. Glass, plastic, or waxed paper sample cups with 50 mL capacity to hold soil and solution for pH measurements
4. Volume dispensers for adding 10 mL deionized water and 10 mL Mehlich buffer to soil
5. Stirring apparatus

6. pH meter with reproducibility to at least 0.05 pH units
7. Glass pH electrode with an internal reference element or a separate reference electrode

## Reagents

1. *Mehlich buffer*: Following are directions for making 2 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The buffer contains 0.043 *M* acetic acid, 0.034 *M* triethanolamine, 0.8 *M* ammonium chloride, 0.08 *M* barium chloride, and 0.08 *M* disodium glycerophosphate with a pH of 6.60.
   a. Add about 1500 mL of deionized water to a 2-L volumetric flask.
   b. Add 5.0 mL of glacial acetic acid ($CH_3COOH$) and stir.
   c. Add 9.0 mL of triethanolamine (TEA; $(HOCH_2CH_2)_3N$) and stir. For ease of delivery, 18 mL of 50% (v/v) TEA can be added.
   d. Add 86 g of ammonium chloride ($NH_4Cl$) and stir to dissolve.
   e. Add 40 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) and stir to dissolve.
   f. In a separate container, add 36 g of glycerophosphate disodium salt hydrate ($Na_2C_3H_5(OH)_2PO_4 \cdot x\ H_2O$ **)** in about 400 mL of deionized water and stir to dissolve. After dissolution, add this solution to the 2-L volumetric flask. The chemical with a mixture of alpha and beta forms is less expensive and as suitable as the pure beta form.
   g. Allow the solution to cool and bring solution to a volume of 2 L with deionized water.
   h. Dilute an aliquot of the buffer solution with an equal volume of deionized water and measure pH while stirring. The pH should be 6.60. If pH is not 6.60, adjust pH of the Mehlich buffer by dropwise additions of glacial acidic acid to decrease pH or 50% (v/v) triethanolamine to increase pH.

   *pH verification:*
   i. Add 4.024 g of aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) to a 1-L volumetric flask. Add 0.05 *M* HCl to dissolve. Bring volume to 1 L with 0.05 *M* HCl. This solution is 0.05 *N* HCl + 0.05 *N* $AlCl_3$.
   j. Add 10 mL of buffer to a mixture of 10 mL deionized water and 10 mL of 0.05 *N* HCl + 0.05 *N* $AlCl_3$. The pH of this mixture should be 4.1±0.05.
2. *Modified-Mehlich buffer*: Follow the directions for making 2 L of Mehlich buffer except for step e. Add 24 g of calcium chloride dihydrate ($CaCl_2 \cdot 2\ H_2O$) in place of barium chloride dihydrate.

## Procedure

1. Measure 10 $cm^3$ of processed soil (dried, < 2 mm) into a 50-mL sample cup.
2. Follow the procedure for making a 1:1 soil-water pH measurement (see Chapter 3.2).
3. Add 10 mL of Mehlich buffer solution to the soil and water mixture and gently swirl for 5 s. Let mixture set for 30 min.
4. Place electrode in the soil slurry and measure soil-buffer pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for robotic pH instruments (see Chapter 3.2).

## Analytical Performance

Range and Sensitivity

1. The measurement sensitivity is 0.4 meq (100 $cm^3$ soil)$^{-1}$ of soil acidity per 0.1 pH unit decrease. This is equivalent to 339 lbs $acre^{-1}$ $CaCO_3$ for a mineral soil with processed soil density of 1.18 g $cm^{-3}$ and 2 million pounds of soil per acre. The lowest soil-buffer pH that can be accurately determined is 4.0. Thus, the range of acidity that can be measured is 0 to 10.4 meq (100 $cm^3$ soil)$^{-1}$. This is equivalent to 0 to 4.4 tons $acre^{-1}$ $CaCO_3$ for a mineral soil with processed soil density of 1.18 g $cm^{-3}$ and 2 million pounds of soil per acre.
2. Soil-buffer pH measurements can be made to the nearest 0.1 or 0.01 pH unit. If measurements are made to the nearest 0.01 pH unit, pH can be rounded to 0.1 pH units before calculating acidity or reporting soil-buffer pH to clients.

Precision and Accuracy

1. Typical measurements of intralaboratory precision for Mehlich soil-buffer pH are shown in Table 1.

*Table 1. Example intralaboratory variations for soil-buffer pH using the Mehlich buffer on four different soils. Measurements were taken on different days.*

| Soil ID | Number of measurements | Soil-buffer pH Mean | Soil-buffer pH Standard deviation |
|---|---|---|---|
| X | 63 | 6.41 | 0.02 |
| A | 63 | 6.30 | 0.02 |
| E | 63 | 5.34 | 0.07 |
| H | 63 | 6.43 | 0.04 |

## Calculations and Interpretation

1. Soil acidity measured from the depression of soil-buffer pH from the initial buffer pH of 6.6 is determined from the following equation.

   Buffer pH acidity = (6.6 – soil-buffer pH) × 4 = meq $H^+$ (100 $cm^3$)$^{-1}$ [1]

   The value of 6.6 is the pH of the Mehlich buffer. For every 0.1 pH unit drop from the initial pH of 6.6, 0.4 meq (100 $cm^3$)$^{-1}$ of acidity has reacted with the buffer.
2. Lime requirement to attain a certain target pH is determined with the following equation.

   Lime requirement (tons $acre^{-1}$) =
   Buffer pH acidity × [(target pH – soil pH) ÷ (6.6 – soil pH)] [2]

   Target pH and soil pH refer to pH measured in 1:1 soil/water mixture. The lime requirement refers to agricultural limestone with at least 90% calcium carbonate equivalence with 90%

passing a 20 mesh sieve and 25% passing a 100 mesh sieve for calcitic or marl limestone, or 90% passing a 20 mesh sieve and 35% passing a 100 mesh sieve for dolomitic limestone.

3. At the North Carolina Department of Agriculture and Consumer Services, lime requirement is determined from Eq. [2] with target pH determined for individual crops and soil class. Soil classes include mineral, mineral-organic, and organic which are determined with a humic matter test (see Chapter 5.5). Target soil pH levels for corn and cotton on mineral soils are 6.0 and 6.2, respectively. General target pH levels for mineral-organic and organic soils are 5.5 and 5.0, respectively. Target soil pH for other combinations of crops and soil classes are presented in Hardy et al. (2012). Due to difficulty in spreading low rates of lime, the minimum lime recommendation given is 0.3 tons $acre^{-1}$.
4. Residual lime in the soil from a previous application in the past 6 to 12 months can be subtracted from lime requirement determined by Eq. [2]. For mineral soils, 8% of lime is assumed to react for each month that elapsed since application. For mineral-organic and organic soils, 16% of lime is assumed to react for each month that elapsed since application. Residual lime in the soil is calculated from the following equation where FACTOR is equal to 0.08 for mineral soils and 0.16 for mineral-organic and organic soils.

   Residual lime (tons $acre^{-1}$) =
   previous lime rate − previous lime rate × (months since application × FACTOR) [3]

5. The modified-Mehlich buffer is used at land grant University laboratories in Pennsylvania, Maine, and Virginia. Lime requirement in these states were determined from a calibration of soil-buffer pH with lime requirement to various target pH values from incubations of soil and lime in the laboratory (Hoskins and Erich, 2008; Wolf et al., 2008). Lime requirement from these various laboratories are very similar to lime requirement from Eq. [2] used in North Carolina (Wolf et al., 2008).

## Effects of Storage

1. Air-dried soil can be stored indefinitely without affecting Mehlich buffer pH measurement.
2. The Mehlich buffer with $BaCl_2$ has an indefinite storage period. The modified-Mehlich buffer with $CaCl_2$ has a shelf life of about 1 to 2 weeks at room temperature due to growth of bacteria or fungi (Nathan et al., 2012).
3. The pH meter and electrodes should be maintained and stored according to manufacturer instructions.

## Safety and Disposal

1. Use proper precautions and personal protective equipment, such as lab coat, gloves, and eye protection, when preparing solutions and conducting analyses.
2. Barium in the Mehlich buffer is defined as a hazardous chemical by the Resource Conservation and Recovery Act due to toxicity (USEPA, 1980a). A laboratory generating more than 100 kg of material in a month is considered a hazardous waste generator that needs to follow hazardous waste disposal protocols defined by the US Environmental Protection Agency (USEPA, 1980b).

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*Chapter 3.7*

# SMP Buffer for Lime Requirement

*F.J. Sikora*

## Application and Principle

The SMP buffer method was developed for lime requirement determination on Ohio soils in 1961 for silt loam soils with a wide range of extractable Al. Prior to 1961, the Woodruff buffer method (Woodruff, 1948) was observed to underestimate lime requirement for Ohio soils with appreciable extractable Al (McLean et al., 1958). Shoemaker, McLean and Pratt developed the SMP buffer method to remedy this problem (Shoemaker et al., 1961). The SMP buffer is added to a soil/water slurry after measurement of soil pH. The SMP buffer has an initial pH of 7.5. Bases in the buffer react with soil acidity to reduce the pH from 7.5 to some measured soil-buffer pH. The greater a decline in soil-buffer pH from an initial value of 7.5, the greater the lime requirement for neutralizing soil acidity. Calibration of soil-buffer pH to lime requirement for achieving various target soil pH values were determined from incubating Ohio soils with varying amounts of $CaCO_3$ (Shoemaker, 1959; Shoemaker et al., 1961).

## Equipment and Apparatus

1. Soil scoop with 4.25 $cm^3$ capacity if measuring soil by volume
2. Analytical balance with 0.01 g resolution if measuring soil by weight
3. Analytical balance with 0.01 g resolution for making SMP buffer
4. Glass, plastic, or waxed-lined paper cups with 50 mL for holding soil and solution for pH measurement
5. Holding rack for pH cups
6. Volume dispensers for adding 5 mL deionized water and 10 mL SMP buffer to soil
7. Manual pH meter or robotic pH analyzer
8. Glass pH electrode with an internal reference element or a separate reference electrode
9. Reciprocating shaker capable of 180 rpm

## Reagents

1. *SMP buffer*: Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The solution contains 0.013 *M* p-nitrophenol, 0.015 *M* potassium chromate, 0.36 *M* calcium chloride, 0.0126 *M* calcium acetate, and 0.019 *M* triethanolamine with a pH of 7.5.
   a. Add 800 mL deionized water to a container.
   b. Add 1.8 g p-nitrophenol ($O_2NC_6H_4OH$) and stir to dissolve.
   c. Add 3 g potassium chromate ($K_2CrO_4$) and stir to dissolve.
   d. Add 53.1 g calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and stir to dissolve.
   e. Add 2 g calcium acetate ($Ca(CO_2CH_3)_2$) and stir to dissolve.

f. Add 2.5 mL triethanolamine (TEA; $(HOCH_2CH_2)_3N$) and dissolve. For ease of delivery, 5 mL of 50% (v/v) TEA can be added.
g. Bring solution volume to 1 L with deionized water.
h. Adjust the pH of the solution to 7.5 with dropwise addition of 40% (w/w) NaOH or 50% (v/v) HCl.

## Procedure

1. Weigh or scoop 5 g of processed soil (dried, < 2 mm) to a sample cup. A scoop volume of 4.25 $cm^3$ will measure 5 g of processed soil with a density of 1.18 g $cm^{-3}$.
2. Follow the procedure for making a 1:1 soil/water pH measurement (see Chapter 3.2).
3. Add 10 mL of SMP buffer.
4. Shake the soil/water/buffer solution on an end-to-end shaker for 10 min and let set for 30 min after shaking. Alternatively, shake for 15 min and let set for 15 min after shaking. If using an automated pH analyzer, stir the buffer with soil vigorously and allow solution to set for at least 30 min.
5. Place electrode in the soil slurry to measure soil-buffer pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for robotic pH instruments (see Chapter 3.2).

## Analytical Performance

Range and Sensitivity

1. Soil-buffer pH is most often within a range from 6.3 to 7.5. For very acidic soils, the soil-buffer pH can be as low as 5.3.
2. Meters typically provide pH measurements to the nearest 0.1 or 0.01 pH unit. If measurements are made to the nearest 0.01 pH unit, pH can be rounded to 0.1 pH units before reporting to clients.

Precision and Accuracy

1. Typical measurements of intralaboratory precision for SMP soil/buffer pH are shown in Table 1.

Interferences

1. The SMP soil-buffer pH reaches stability quicker than pH measured in deionized water due to the pH buffering capacity of the SMP buffer and the presence of electrolytes in the buffer. Differences in pH may still occur with electrode placed in a soil-slurry or in the supernatant after the soil has settled. To avoid this variability in pH, it is important to stir the soil slurry right before measurement.
2. The junction potential of electrodes can become clogged during storage or after repeated measurements. Make sure to clean electrodes periodically to ensure proper measurements. Replace electrodes when they consistently fail to measure pH of calibration buffers or when measurement of quality control samples consistently show more error than expected.

*Table 1. Example intralaboratory variations for soil-buffer pH using the SMP buffer on four different soils. Measurements were taken on different days using a LabFit robotic pH instrument.*

| Soil texture | Number of measurements | Soil-buffer pH Mean | Soil-buffer pH Standard deviation |
|---|---|---|---|
| Silt loam | 10 | 6.04 | 0.05 |
| Silt loam | 10 | 6.27 | 0.04 |
| Sandy loam | 10 | 6.76 | 0.03 |
| Sand | 10 | 7.31 | 0.03 |

3. The glass bulb on the electrode scratches with use in a soil slurry. These scratches slow the pH response time. Replace electrode when measurement response is sluggish.

## Interpretation

1. Lime requirement is obtained from the SMP soil-buffer pH. The SMP soil-buffer pH was calibrated to lime requirement for achieving a target soil-water pH of 6.0, 6.4, or 6.8 in Ohio soils as shown in Table 2 (Shoemaker, 1959; Shoemaker et al., 1961). Lime requirement is shown as pure $CaCO_3$ for a 6 inch plow depth. Laboratories may have different recommendations from calibrations specific to soils in their region.
2. To adjust to a rate of agricultural limestone that is less effective than pure $CaCO_3$, multiply the $CaCO_3$ rate in Table 2 by 100 ÷ (percent effectiveness of agricultural limestone). A common percent effectiveness of agricultural limestone is 67% which results in a multiplication factor of 1.5.
3. To adjust lime requirement to a soil depth other than 6 inches, multiply the agricultural limestone rate by soil depth ÷ 6. For a soil depth of 8 inches, the multiplication factor would be 1.33.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil-buffer pH measurement provided they are stored in an ammonia free environment or in a tightly sealed container.
2. The pH meter and electrodes should be maintained and stored according to manufacturer instructions.

## Safety and disposal

1. The p-nitrophenol and potassium chromate in the SMP buffer are defined as hazardous chemicals by the Resource Conservation and Recovery Act due to toxicity (USEPA, 1980a). A laboratory generating more than 100 kg of material in a month is considered a hazardous waste generator that needs to follow hazardous waste disposal protocols defined by the US Environmental Protection Agency (USEPA, 1980b).

*Table 2. Lime requirement with pure $CaCO_3$ to raise soil-water pH to target values of 6.0, 6.4, and 6.8 based on SMP soil-buffer pH.*

| | $CaCO_3$ (tons $acre^{-1}$) to increase soil-water pH to the following values | | |
|---|---|---|---|
| Soil-buffer pH | pH 6.0 | pH 6.4 | pH 6.8 |
| 6.7 | 1.0 | 1.2 | 1.4 |
| 6.6 | 1.4 | 1.7 | 1.9 |
| 6.5 | 1.8 | 2.2 | 2.5 |
| 6.4 | 2.3 | 2.7 | 3.1 |
| 6.3 | 2.7 | 3.2 | 3.7 |
| 6.2 | 3.1 | 3.7 | 4.2 |
| 6.1 | 3.5 | 4.2 | 4.8 |
| 6.0 | 3.9 | 4.7 | 5.4 |
| 5.9 | 4.4 | 5.2 | 6.0 |
| 5.8 | 4.8 | 5.7 | 6.5 |
| 5.7 | 5.2 | 6.2 | 7.1 |
| 5.6 | 5.6 | 6.7 | 7.7 |
| 5.5 | 6.0 | 7.2 | 8.3 |
| 5.4 | 6.5 | 7.7 | 8.9 |
| 5.3 | 6.9 | 8.2 | 9.4 |

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*Chapter 3.8*

# Adams-Evans, Modified Adams-Evans, and Moore-Sikora Buffers for Lime Requirement

*G. Huluka, K.P. Moore, and F.J. Sikora*

## Application and Principle

Most soils of the Southeastern US are acidic and amelioration with lime application has been a common practice for crop production. The Adams-Evans lime buffer solution was developed in Alabama on coarse-textured soils with low clay content dominated by kaolinite and sesquioxide soil minerals (Adams and Evans, 1962; Franklin et al., 1998). These soils have relatively low cation exchange capacity of less than 13 $cmol_c\ kg^{-1}$, and usually need less than 5 tons $acre^{-1}$ lime to reach target pH values between 6 and 7.

Buffers developed by Morgan (1930), Mehlich (1938), Woodruff (1948), Shoemaker et al. (1961), and Adams and Evans (1962) contained p-nitrophenol because it provided good pH buffering in the pH range of most acidic soils. However, p-nitrophenol has been classified as a hazardous chemical (USEPA, 1980a) and alternative buffers have been developed with p-nitrophenol replaced by chemicals that buffer pH in a similar pH range. These alternate buffers produce soil-buffer pH analogous to that from Adams-Evans buffer. The modified Adams-Evans buffer was developed by replacing the p-nitrophenol with monobasic potassium phosphate ($KH_2PO_4$) that has a pH buffering capacity similar to p-nitrophenol (Huluka, 2005). The Moore-Sikora buffer was developed by replacing p-nitrophenol with 3-(N-morpholino)propanesulfonic acid (MOPS) and 2-(N-morpholino)ethanesulfonic acid hydrate (MES) which also buffers pH in a range similar to p-nitrophenol (Moore and Sikora, 2007; Sikora and Moore, 2008).

The procedures for using the Adams-Evans, modified Adams-Evans, and Moore-Sikora buffers are presented. Since the modified Adams-Evans and Moore-Sikora buffers produce the same soil-buffer pH as the Adams-Evans buffer, the calculations and interpretation of data for lime requirement determination are the same for these three methods. The primary difference amongst the buffer methods is the chemical content of the buffers.

## Equipment and Apparatus

1. Soil scoop with 10 or 12 $cm^3$ volume and leveling rod
2. Analytical balance with 0.01 g resolution for making buffer
3. Glass, plastic, or waxed-lined paper cups with 50 to 70 mL capacity for holding soil and solution for pH measurement
4. Holding rack for pH cups
5. Dispensers for adding deionized water and buffer to soil
6. Manual pH meter or robotic pH analyzer
7. Glass pH electrode with an internal reference element or a separate reference electrode

## Reagents

1. *Standardization buffers at or near pH 4, 7, and 10*
2. *Adams-Evans buffer*: Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The buffer contains 1 *M* potassium chloride, 0.14 *M* p-nitrophenol, and 0.24 *M* boric acid at a pH of 8.00.
   a. Weigh 74 g of potassium chloride (KCl).
   b. Weigh 20 g of p-nitrophenol ($O_2NC_6H_4OH$).
   c. Add the weighed components in the previous two steps to a 1-L volumetric flask. Add approximately 700 mL of deionized water. Stir until completely dissolved.
   d. Weigh 15 g of boric acid ($H_3BO_3$).
   e. Weigh 10.5 g of potassium hydroxide (KOH).
   f. Add the weighted components in the previous two steps to the 1-L volumetric flask containing potassium chloride and p-nitrophenol. Mix until completely dissolved.
   g. Adjust pH to 8.00 ± 0.01 using either dilute KOH or HCl.
   h. Bring solution to 1 L volume with deionized water.
3. *Modified Adams-Evans buffer*: Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The buffer contains 1 *M* potassium chloride, 0.22 *M* potassium phosphate, and 0.24 *M* boric acid at a pH of 8.00.
   a. Weigh 74 g of potassium chloride (KCl).
   b. Weigh 30 g of potassium phosphate monobasic ($KH_2PO_4$).
   c. Weigh 10.5 g of potassium hydroxide (KOH).
   d. Add the weighed components in the previous three steps to a 1-L volumetric flask. Add approximately 700 mL of deionized water and stir until completely dissolved.
   e. Add 15 g of boric acid ($H_3BO_3$) and stir to dissolve.
   g. Adjust pH to 8.00 ± 0.01 using either dilute KOH or HCl.
   h. Bring solution to 1 L volume with deionized water.
4. *Moore-Sikora buffer*: Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes of the buffer. The buffer contains 1 *M* potassium chloride, 0.035 *M* MES, 0.13 *M* MOPS, and 0.21 *M* boric acid at a pH of 7.92.
   a. Weigh 74 g of potassium chloride (KCl).
   b. Weigh 7.43 g of 2-(N-Morpholino)ethanesulfonic acid hydrate (MES; $C_6H_{13}NO_4S \cdot xH_2O$).
   c. Weigh 27.4 g 3-(N-Morpholino)propanesulfonic acid (MOPS; $C_7H_{15}NO_4S$).
   d. Weigh 13.1 g boric acid ($H_3BO_3$).
   e. Weigh 11.2 g potassium hydroxide (KOH).
   f. Add each of the weighed components to a 1-L volumetric flask.
   g. Bring volume to 1 L with deionized water and stir the solution overnight.
   h. Adjust the pH to 7.92 ± 0.013 using dropwise addition of concentrated KOH or HCl.
   i. The pH of a 1:1 buffer/water mixture should be 8.00 ± 0.01. If the pH of the 1:1 mixture is not 8.00 ± 0.01, adjust the pH of the original solution using dropwise additions of concentrated KOH or HCl until the pH of a 1:1 buffer/water mixture is at the desired pH of 8.00 ± 0.01.

## Procedure

Adams-Evans and Modified Adams-Evans Buffers

1. Measure 10 $cm^3$ processed soil (dried, < 2 mm) into the sample container.
2. Calibrate the pH meter to pH 8 using a 1:1 buffer/water mixture. Alternatively, the pH meter can be calibrated with pH 4 and 7 buffer solutions and the pH of a 1:1 buffer/water mixture measured. If the measured pH is different than 8.00, adjust the pH of soil-buffer pH measurements by the difference.
3. Add 10 mL of deionized water to soil. Stir sample and water with a glass rod and allow sample to set for 30 min to 1 h prior to taking a soil-water pH measurement while stirring with a mechanical stirrer.
4. Add 10 mL of the buffer to the soil-water mixture. Stir the soil-water-buffer mixture vigorously with a stir bar or mechanical stirrer.
5. After 30 min, make a soil-buffer pH measurement while stirring with a mechanical stirrer.

Moore-Sikora buffer

1. Measure 12 $cm^3$ of processed soil (dried, < 2 mm) into the sample container. The average density of coarse-textured soil tested at Clemson University is 1.25 g $cm^{-3}$. Therefore, 12 $cm^3$ is assumed to measure 15 g. After addition of water and buffer, the resultant ratio of soil/water/buffer is 1:1:1 (w/v/v).
2. Calibrate the pH meter using pH 4 and 7 calibration buffers. Measure pH of a 1:1 buffer/water mixture. If the pH is different from 8.00, the pH of the Moore-Sikora buffer can be adjusted with KOH or HCl. Alternatively, the pH difference can be used to correct soil-buffer pH measurements. For example, if the pH of the 1:1 mixture was 8.02, a correction value of 0.02 is subtracted from all soil-buffer pH measurements.
3. Add 15 mL of deionized water to soil. Stir sample and water with a glass rod and allow sample to set for 30 min to 1 h prior to taking a soil/water pH measurement.
4. Add 15 mL of the buffer to the soil/water mixture. Stir the soil/water/buffer mixture vigorously with a stir bar or mechanical stirrer.
5. Other soil weights and volumes for water and buffer may be used as long as the ratio remains 1:1:1 (w/v/v).
6. After 30 min, the soil, water, and buffer mixture is stirred again and a soil-buffer pH measurement is taken. Buffer pH measurements can be made with or without stirring during the measurement, however, it is important to stir the soil slurry before the measurement is taken.

## Calculations

1. The following equation defines the relationship between soil-water pH and $H_{sat}$ which is the fraction of the cation exchange capacity (CEC) occupied by exchangeable acidic cations (Adams and Evans, 1962).

   $$\text{Soil-water pH} = 7.79 - 5.55 \times (\text{Hsat}) + 2.27 \times (\text{Hsat})^2$$

   The fraction of exchangeable acidic cations at an initial soil-water pH is defined as $Hsat_1$. The fraction of exchangeable acidic cations at a desired soil-water pH is defined as $Hsat_2$.

Values for $Hsat_1$ and $Hsat_2$ are determined by using the quadratic formula on the above equation.

2. A slightly different relationship was observed between pH and fraction of CEC occupied by exchangeable acidic cations in the development of the modified Adams-Evans buffer as shown below (Huluka, 2005).

   Soil-water pH = $7.60 - 5.71 \times (Hsat) + 2.99 \times (Hsat)^2$

3. The initial pH of a 1:1 water/buffer mixture is 8.00. The soil-buffer pH will be some value less than 8.00 due to soil acidity. A pH decrease of 0.01 is caused by 0.08 $cmol_c\ kg^{-1}$ soil acidity reacting with the buffer. Therefore, the exchangeable acidity can be determined by the following.

   a. Exchangeable acidity ($cmol_c\ kg^{-1}$) =
      0.08 $cmol_c\ kg^{-1}$ ÷ (0.01 pH unit change) × (8.0 – soil-buffer pH)

   b. Exchangeable acidity ($cmol_c\ kg^{-1}$) = 8 × (8.0 – soil-buffer pH)

4. The CEC can be presented as exchangeable acidity in $cmol_c\ kg^{-1}$ divided by the fraction of CEC containing exchangeable acidity at the initial pH of the soil ($Hsat_1$).

   a. CEC = exchangeable acidity ÷ ($Hsat_1$)

   b. CEC = 8 × (8.0 – soil-buffer pH) ÷ ($Hsat_1$)

5. The acidity to be neutralized in $cmol_c\ kg^{-1}$ is related to the CEC and the exchangeable acidities of the initial and desired soil pH values by the following equations.

   a. Acidity to be neutralized ($cmol_c\ kg^{-1}$) = CEC × ($Hsat_1 - Hsat_2$)

   b. Acidity to be neutralized ($cmol_c\ kg^{-1}$) =
      8 × (8.0 – soil-buffer pH) ÷ ($Hsat_1$) × ($Hsat_1 - Hsat_2$)

6. Acidity to be neutralized ($cmol_c\ kg^{-1}$) is converted to lbs $acre^{-1}$ limestone ($CaCO_3$) required by using 50 g $eq^{-1}$ as the equivalent weight of $CaCO_3$ and assuming 2 million pounds of soil in one acre at a 6 inch depth. The conversion leads to multiplying the acidity to be neutralized ($cmol_c\ kg^{-1}$) by 1000 to obtain lbs $acre^{-1}$ $CaCO_3$ as follows.

   $CaCO_3$ (lbs $acre^{-1}$) = 1000 × 8 × (8.0 – soil-buffer pH) ÷ ($Hsat_1$) × ($Hsat_1 - Hsat_2$)

7. The limestone required from the previous equation is based on pure $CaCO_3$. A multiplication factor of 100 divided by percent limestone effectiveness can be used to determine the rate of agricultural limestone as shown in the following equation. On average, agricultural limestone is about 67% as effective as $CaCO_3$. For this percent effectiveness, the multiplication factor is 1.5.

Agricultural limestone (lbs $acre^{-1}$) =
$8000 \times (8.0 - \text{soil-buffer pH}) \div (Hsat_1) \times (Hsat_1 - Hsat_2) \times$
(100 ÷ % limestone effectiveness)

8. Another multiplication factor can be used if a soil depth other than 6 inches is to be treated. For example, a soil depth of 8 inches rather than 6 inches results in a multiplication factor of 8/6 or 1.33. Agricultural limestone application for a different soil depth may be calculated as follows.

Agricultural limestone (lbs $acre^{-1}$) =
$8000 \times (8.0 - \text{soil-buffer pH}) \div (Hsat_1) \times (Hsat_1 - Hsat_2) \times$
(100 ÷ % limestone effectiveness) × (soil depth inches ÷ 6 inches)

## Analytical Performance

Range and Sensitivity

1. The method is reliable for soils with levels of exchangeable acidity less than 8 $cmol_c\ kg^{-1}$. If the soil-buffer pH is less than 7.0, the soil contains more than 8 $cmol_c\ kg^{-1}$ of exchangeable acidity and exceeds the method's limit for accurate lime requirement determination.
2. The lime requirement may be made to the nearest 100 or 500 lbs $acre^{-1}$.
3. The soil-water pH can be determined to the nearest 0.1 pH unit.
4. Due to the strong pH buffering of the buffers, measurements of soil-buffer pH to 0.01 decimal places are required.

Precision and Accuracy

1. Typical measurements of precision for modified Adams-Evans and Moore-Sikora soil-buffer pH are shown in Table 1.

*Table 1. Example intralaboratory variations for soil-buffer pH using the modified Adams-Evans and Moore-Sikora buffers. Measurements were taken on different days using a LabFit robotic pH instrument.*

| Soil texture | Number of measurements | Soil-buffer pH Mean | Soil-buffer pH Standard deviation |
|---|---|---|---|
| | Modified Adams-Evans buffer | | |
| Sandy Loam | 10 | 7.72 | 0.02 |
| Loam | 10 | 7.66 | 0.03 |
| | Moore-Sikora buffer | | |
| Clay Loam | 20 | 7.76 | 0.02 |
| Loamy Sand | 20 | 7.42 | 0.02 |

Interferences

1. Soil-buffer pH reaches stability quicker than pH measured in deionized water due to the strong pH buffering capacity of the buffer solution and the presence of electrolytes in the buffer. Differences in pH may still occur with electrode placed in a soil-slurry or in the supernatant after the soil has settled. To avoid this variability in pH, it is important to stir the soil slurry right before measurement.
2. The junction potential of electrodes can become clogged during storage or after repeated measurements. Make sure to clean electrodes periodically to ensure proper measurements. Replace electrodes when they consistently fail to measure pH of calibration buffers or when measurement of quality control samples consistently show more error than expected.
3. The glass bulb on the electrode scratches with use in a soil slurry. These scratches slow the pH response time. Replace electrode when measurement response is sluggish.

## Interpretation

1. Lime requirement can be determined from soil-water pH, soil-buffer pH, target soil-water pH, lime quality, and soil depth using the equations in the calculation section. The equations are valid for desired soil-water pH of 6.5 or less.
2. Target pH varies according to the crop grown and generally ranges from 6.0 to 6.5. Minimum and maximum limits can be placed on lime recommendations.
3. Lime requirement from Clemson University using Adams-Evans and Moore-Sikora buffers for various soil-water and soil-buffer pH values with a target soil-water pH of 6.5, treatment soil depth of 8 inches, and lime quality of 67% effectiveness are shown in Table 2.
4. Lime requirement from Auburn University using modified Adams-Evans buffer for various soil-water and soil-buffer pH values using a plow depth of 8 inches, a target pH of 6.5, and a lime quality that is 67% as effective as pure $CaCO_3$ are shown in Table 3.
5. Lime may not be recommended if soil-water pH is slightly less than the target pH to avoid very small lime applications and since crop growth is not adversely affected by soil-water pH slightly below the target pH. For example, a trigger soil-water pH of 6.3 may be used where lime is only recommended to raise soil-water pH to 6.5 if soil-water pH is less than or equal to 6.3.
6. Minimum and maximum lime recommendations should be considered based on lime application limitations. Auburn University uses a minimum of 2000 lbs $acre^{-1}$ and a maximum of 9000 lbs $acre^{-1}$ for agronomic crops. Clemson University uses a minimum of 2000 lbs $acre^{-1}$ for alfalfa, a minimum of 1000 lbs $acre^{-1}$ for all other agronomic crops, and a maximum of 10,000 lbs $acre^{-1}$ on all agronomic crops.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil-buffer pH measurement provided they are stored in an ammonia free environment or in a tightly sealed container.
2. The pH meter and electrodes should be maintained and stored according to manufacturer instructions.

*Table 2. Lime requirement from Clemson University using Adams-Evans or Moore-Sikora buffers for treating 8 inch soil depth to a target soil-water pH of 6.5 using 67% effective agricultural limestone.*

| | Required Limestone (lbs acre$^{-1}$) using Adams-Evans or Moore-Sikora buffers | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Soil-buffer | Soil-water pH | | | | | | | | | |
| pH | 6.3 | 6.1 | 5.9 | 5.7 | 5.5 | 5.3 | 5.1 | 4.9 | 4.7 | 4.5 |
| 7.90 | 200 | 400 | 600 | 700 | 800 | 900 | 1,000 | 1,000 | 1,100 | 1,200 |
| 7.80 | 500 | 900 | 1,200 | 1,400 | 1,600 | 1,800 | 2,000 | 2,100 | 2,200 | 2,400 |
| 7.70 | 700 | 1,300 | 1,700 | 2,100 | 2,400 | 2,700 | 2,900 | 3,100 | 3,300 | 3,600 |
| 7.60 | 1,000 | 1,700 | 2,300 | 2,800 | 3,200 | 3,600 | 3,900 | 4,200 | 4,500 | 4,800 |
| 7.50 | 1,200 | 2,200 | 2,900 | 3,500 | 4,000 | 4,500 | 4,900 | 5,200 | 5,600 | 5,900 |
| 7.40 | 1,500 | 2,600 | 3,500 | 4,200 | 4,800 | 5,400 | 5,900 | 6,300 | 6,700 | 7,100 |
| 7.30 | 1,700 | 3,000 | 4,100 | 4,900 | 5,700 | 6,300 | 6,800 | 7,300 | 7,800 | 8,300 |
| 7.20 | 2,000 | 3,500 | 4,700 | 5,600 | 6,500 | 7,200 | 7,800 | 8,400 | 8,900 | 9,500 |
| 7.10 | 2,200 | 3,900 | 5,200 | 6,300 | 7,300 | 8,100 | 8,800 | 9,400 | 10,000 | 10,600 |
| 7.00 | 2,400 | 4,300 | 5,800 | 7,100 | 8,100 | 9,000 | 9,800 | 10,400 | 11,100 | 11,800 |

*Table 3. Lime requirement from Auburn University using modified Adams-Evans buffer for treating 8 inch soil depth to a target soil-water pH of 6.5 using 67% effective agricultural limestone.*

| | Required Limestone (lbs acre$^{-1}$) using modified Adams-Evans buffer | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Soil-buffer pH | Soil-water pH 6.3 | 6.1 | 5.9 | 5.7 | 5.5 | 5.3 | 5.1 | 4.9 |
| 7.90 | 300 | 500 | 700 | 800 | 900 | 1,000 | 1,100 | 1,200 |
| 7.80 | 600 | 1,000 | 1,300 | 1,600 | 1,800 | 2,000 | 2,200 | 2,400 |
| 7.70 | 900 | 1,500 | 2,000 | 2,400 | 2,700 | 3,000 | 3,300 | 3,600 |
| 7.60 | 1,100 | 2,000 | 2,600 | 3,200 | 3,600 | 4,000 | 4,400 | 4,800 |
| 7.50 | 1,400 | 2,500 | 3,300 | 3,900 | 4,500 | 5,000 | 5,400 | 6,000 |
| 7.40 | 1,700 | 3,000 | 3,900 | 4,700 | 5,400 | 6,000 | 6,500 | 7,200 |
| 7.30 | 2,000 | 3,500 | 4,600 | 5,500 | 6,300 | 7,000 | 7,600 | 8,400 |
| 7.20 | 2,300 | 4,000 | 5,300 | 6,300 | 7,200 | 8,000 | 8,700 | 9,600 |
| 7.10 | 2,600 | 4,400 | 5,900 | 7,100 | 8,100 | 9,000 | 9,800 | 10,800 |
| 7.00 | 2,800 | 4,900 | 6,600 | 7,900 | 9,000 | 10,000 | 10,900 | 12,000 |

## Safety and disposal

1. The p-nitrophenol in the Adams-Evans buffer is defined as a hazardous chemical by the Resource Conservation and Recovery Act due to toxicity (USEPA, 1980a). A laboratory generating more than 100 kg of material in a month is considered a hazardous waste generator that needs to follow hazardous waste disposal protocols defined by the US Environmental Protection Agency (USEPA, 1980b).
2. The chemicals used in the modified Adams-Evans and Moore-Sikora buffer methods pose no health or safety risk and therefore can be stored and disposed of according to routine laboratory procedures.

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*Unit 4*

# Soil Extractable Plant Nutrients

*Chapter 4.1*

# Introduction to Soil Extractable Plant Nutrients

*R. Miller and J.L. Oldham*

There are seventeen chemical elements considered essential for plants to complete their life cycle. These plant nutrients are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, copper, zinc, manganese, boron, molybdenum, iron, chlorine, and nickel. Carbon, hydrogen, and oxygen are supplied to the plant through photosynthesis. The remaining nutrients are absorbed by plant roots from the soil. Insufficient nutrients may limit or end plant growth, decrease crop yields, and lower profitability. Excessive nutrients may limit or end plant development, decrease yields, lower profits, and increase environmental risks.

In nature, nutrients cycle from plants to soils and back again through constant turnover. Human management in agriculture alters the natural cycling of nutrients where harvested crop biomass removes nutrients from the field. Supplemental nutrients may be required to replace nutrients removed by harvested crops or to provide nutrients to optimize growth of crop hybrids developed for high yields. Fertilizers, manures, legumes, and other sources may provide supplemental nutrition for growing crops.

Plant essential nutrients are classified as primary macronutrients, secondary macronutrients, and micronutrients. The terminology defining the type of nutrient corresponds to the concentration of nutrient in the plant required for optimum growth. Good technical publications on the biochemical roles of nutrients in plants are provided by Maathuis (2009) and Hansch and Mandel (2009). A good extension publication describing the function of nutrients in plants with photographs of deficiency symptoms is provided by McCauley et al. (2009). A summary of the essential nutrient functions in plants and how plant-available nutrients are tested in soil is provided below.

## Primary Macronutrients

1. *Nitrogen (N)*: Nitrogen is required for protein production and is a key component of the nucleic acids DNA and RNA. It is a component of chlorophyll, which gives the green color to plants and is vital for photosynthesis. Crops typically take up N from soils as nitrate and to a much lesser extent as ammonium. Soil nitrogen in the ammonium form is readily converted to nitrate. Soil nitrates are highly mobile in the soil and are often present at limited quantities as they are lost to leaching or denitrification. The Southeastern US environment is typically warm and humid which allows for rapid conversion of various N forms in soil. Thus, it is difficult to test N in soil at one time in the year and predict N availability to the plant during the growing season. An exception is climates with limited rainfall as occurs in Texas and Oklahoma. Since nitrate loss via leaching or denitrification is minimized with limited rainfall, soil can be tested for nitrate to provide a good estimate on nitrate availability to the plant. Another exception is testing soil for nitrate in corn production before sidedressing N fertilizer. This test is commonly referred to as the presidedress nitrate test (PSNT) and has the potential of adequately predicting the amount of sidedress N to apply.

2. *Phosphorus (P)*: Phosphorus enables plants to convert solar energy into chemical energy which is used to synthesize sugars, starches, and proteins. Phosphorus is relatively immobile in soils. Thus, plants may have a P deficiency early in the season since the volume of soil roots occupy and actively absorb nutrients from is small. Phosphorus deficiency can persist if root growth is inhibited through factors such as low soil pH, soil temperature, or soil moisture. Most fertilizer P added to soil reacts with iron, aluminum, and calcium to become insoluble and unavailable to the plant. Therefore, the amount of plant-available phosphorus is much lower than total phosphorus in soil. There are several soil tests that are successful in extracting a portion of the insoluble P that is well correlated to the amount of P utilized by plants.
3. *Potassium (K)*: Plants use K to photosynthesize, transport sugar, move water and nutrients, synthesize protein, and form starch. Adequate plant K improves disease resistance, water stress tolerance, winter hardiness, tolerance to many pests, and uptake of other nutrients. Under good growing conditions, forage crops remove large quantities of potassium from the soil, whereas cereal and legume grain crops remove lower amounts. Potassium uptake is often equal to N uptake and several times more than P uptake. Where levels of soluble K in the soil are high, plants may take up more K than needed and is referred to as "luxury consumption" since it does not increase yields. Potassium mobility is strongly related to soil texture and movement is greatest in soils with high sand content. Potassium is most likely to build up in clay soils, followed by loams and coarse-textured sands. As with P, there are several soil tests proven successful in predicting plant-availability of K in soil.

## Secondary Macronutrients

1. *Sulfur (S)*: Sulfur is a component of some amino acids used in building proteins. Plants need about the same quantity of S as they do P. Sulfur is mobile in soils and can be lost by leaching. Within plants, however, S is immobile. In the past, sulfur was added to agricultural soils in significant quantities from fertilizers containing sulfate salts of the primary macronutrients and from atmospheric deposition with burning of fossil fuels. Lower S concentrations in modern fertilizer materials and reduced atmospheric S from automobiles and industry have caused S deficiencies to be more common than in the past. Soil tests for S are based on extraction of soluble sulfate-S. It is difficult to interpret these test results because a majority of plant-available S can come from mineralization of soil organic matter. Soil tests that determine sulfate-S are more successful in sandy soil or subsoil with low organic matter content.
2. *Calcium (Ca)*: Calcium is a component of the cell wall and stabilizes cell membranes. Calcium deficiencies are usually found in growing points of the plant at the fruit, stem, leaf, and root tips. Calcium deficiency is unusual in the Southeastern US. An exception is peanuts where more calcium is needed by the crop than can be supplied by the soil. Calcium is tested along with P and K with most soil test extractants.
3. *Magnesium (Mg)*: Magnesium is the central part of the chlorophyll molecule where photosynthesis occurs. It also helps the plant metabolize energy and form protein. Soil magnesium deficiencies in pastures may lead to magnesium deficiencies in animals grazing the forage. Magnesium is tested along with P and K with most soil test extractants.

## Micronutrients

1. *Copper (Cu)*: Copper is involved in cell respiration, protein synthesis, seed formation, and chlorophyll production. It is immobile in soil and can accumulate when more is applied than removed by grain or forage biomass. Soil organic matter holds copper tightly.
2. *Zinc (Zn)*: Zinc is necessary for starch formation, protein synthesis, root development, growth hormones, and enzyme systems. As with Cu, Zn is relatively immobile in soils and tends to accumulate. Zinc deficiencies are most common on sandy soils that have low organic matter and soil with high pH and P levels, especially under cool and wet conditions. Zinc deficiency symptoms are evident on small plants as interveinal light striping or a whitish band beginning at the base of the leaf.
3. *Manganese (Mn)*: Manganese is involved in chlorophyll formation, nitrate assimilation, enzyme systems, and iron metabolism. Manganese deficiency is generally caused by high soil pH and Mn toxicity is caused by low soil pH.
4. *Boron (B)*: Boron is involved in sugar and starch balance and translocation, pollination and seed production, cell division, N and P metabolism, and protein formation. Boron, like N and S, is highly mobile, especially in sandy soils. Because of this mobility, B must be added annually for crops sensitive to deficiencies. However, excessive rates of B are detrimental and should be avoided.
5. *Molybdenum (Mo)*: Molybdenum is involved in protein synthesis, legume N fixation, enzyme systems, and N metabolism. Deficiencies of Mo generally occur on acidic soils with high levels of iron and aluminum oxides. Soil pH largely controls the availability of Mo to the plant.
6. *Iron (Fe)*: Iron is used in chlorophyll and protein formation, enzyme systems, respiration, photosynthesis, and energy transfer. Iron deficiency is not common in acid soils that are typical in the Southeastern US. Iron deficiency is more common in calcarerous soil with high pH that results in Fe becoming insoluble. Deficiency can also be caused by an imbalance of metallic ions such as Cu and Mn, and excessive amounts of P.
7. *Chlorine (Cl)*: Chlorine is involved in photosynthesis, water-use efficiency, crop maturity, disease control and sugar translocation. While chloride leaches quite readily in coarse-textured soils, deficiencies are relatively uncommon.
8. *Nickel (Ni)*: Plants require nickel for proper seed germination. Nickel is also a component in urease, which helps convert urea to ammonium. Nickel is a relatively newly defined essential element and specific deficiency symptoms are unclear beyond chlorosis.
9. *Soil Tests for Micronutrients*: Extractants designed specifically for micronutrients include DTPA, DTPA with sorbitol, and hot-water for B. With the advent of inductively couple plasma – atomic emission spectroscopy (ICP-AES), micronutrients became readily analyzed in soil test extractants developed for macronutrients, such as Mehlich-1 and Mehlich-3. Interpretation of micronutrient results is often tenuous since there is limited correlation and calibration data for fertilizer recommendations to be made.

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*Chapter 4.2*

# Mehlich-1

*R. Mylavarapu and R. Miller*

## Application and Principle

The Mehlich-1 extraction method was developed by Mehlich in 1953 to determine the bioavailability of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in soils (Mehlich, 1953) with a particular emphasis on P (Nelson et al., 1953). Mehlich-1 extractable P correlated poorly to crop growth on soils from diverse physiographic regions of North Carolina, calcareous soils, and soils containing phosphate rock (Mehlich, 1978; Mehlich, 1984). However, the method is suitable for determining bioavailable P in acid soils with low cation exchange capacity (CEC) less than 10 $cmol_c\ kg^{-1}$. The Mehlich-1 extracting reagent contains 0.05 *M* HCl and 0.0125 *M* $H_2SO_4$ and was originally referred to as the Double Acid method. The original method had P quantified in the extract via spectrophotometry by reacting P with a molybdate molecule to form a blue-colored complex. The method for analyzing P that is currently performed utilizes inductively coupled plasma-atomic emission spectrometry (ICP-AES). The amount of P and K extracted with Mehlich-1 has been calibrated to quantify the amount of fertilizer P and K needed to maximize crop yields in various regions of the Southeastern US. The method has also been adapted for simultaneous determination of other plant nutrients in the extract using ICP-AES.

## Equipment and Apparatus

1. Soil scoop with 4 $cm^3$ capacity if measuring soil by volume
2. Analytical balance with 0.01 g resolution if measuring soil by weight
3. Analytical balance with 0.01 g resolution for making Mehlich-1 extractant
4. Polyethylene carboy with 20 L capacity
5. Reciprocating mechanical shaker capable of 180 oscillations per minute
6. Volume dispenser to deliver 20 mL of Mehlich-1 extractant
7. Extraction bottles or flasks (50 to 100 mL) with stoppers
8. Filter funnels and vials for receiving filtrates
9. Whatman No. 1 filter paper or equivalent
10. Vortex stirrer if analyzing P via molybdate-blue spectrophotometry
11. Inductively coupled plasma-atomic emission (ICP-AE) spectrometer.

## Reagents

1. *Mehlich-1 extracting solution*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 0.05 *M* HCl and 0.0125 *M* $H_2SO_4$.
   a. Fill a 20-L calibrated plastic carboy with approximately 18 L of deionized water.

b. Add 83 mL of concentrated HCl.
c. Add 14 mL of concentrated $H_2SO_4$.
d. Bring the solution to 20 L with deionized water and thoroughly stir.
e. The pH of the extracting solution is approximately 1.2.

2. *Calibrations Standards*
   a. From commercially purchased standard solutions containing 1,000 mg $L^{-1}$ of each analyte, prepare 1 L of a standard in Mehlich-1 extracting solution containing the highest concentration of each element. This is a calibration standard with the highest concentration of analytes.
   b. Prepare additional calibration standards by diluting the most concentrated calibration standard with Mehlich-1. A recommended concentration range for calibration standards is shown in Table 1 for ICP-AES. If analyzing P via molybdate-blue spectrophotometry, prepare a blank and standards with 1, 2, 5, 10, 15, and 20 mg $L^{-1}$ P.
   c. Cations (K, Ca, Mg, Na, Fe, Mn, Cu, Zn) can be grouped together in the same calibration standards for ICP-AES. Likewise, anion (P, B) analytes can be grouped together. Avoid combining cation and anion analytes in the same standards since precipitation may remove analytes from solution.

*Table 1. Suggested calibration standard concentrations (mg $L^{-1}$) for quantitative analysis of Mehlich-1 soil extracts using ICP-AES.*

| | P | K | Ca | Mg | Na | Fe | Mn | Cu | Zn | B |
|---|---|---|---|---|---|---|---|---|---|---|
| Blank | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Standard 1 | 10 | 10 | 50 | 10 | 10 | 10 | 2 | 2 | 2 | 2 |
| Standard 2 | 25 | 25 | 100 | 50 | 50 | 25 | 5 | 5 | 5 | 5 |
| Standard 3 | 50 | 50 | 500 | 100 | 100 | 50 | 10 | 10 | 10 | 10 |

## Procedure

Extraction

1. Scoop 4 $cm^3$, or weigh 5 g, of processed soil (dried, < 2 mm) and add to an extraction flask.
2. Add 20 mL of Mehlich-1 extracting solution to the flask with a volume dispenser.
3. Alternative scoop and Mehlich-1 volumes can be used as long as the volume of Mehlich-1 to scoop volume of soil has a ratio of 5:1.
4. Add stoppers to flasks and shake soil and Mehlich-1 for 5 min on a reciprocating mechanical shaker with a minimum of 180 oscillations per minute.
5. Filter the suspension using Whatman No. 1 filter paper and collect the filtrate in vials for analysis. Refilter if filtrate is cloudy.

Phosphate Analysis via Molybdate-Blue Spectrophotometry

1. University laboratories in the Southeastern US using the Mehlich-1 method analyze P in the extract with ICP-AES. The method was originally developed with P analysis via molybdate-blue spectrophotometry which can be found in Chapter 4.3.

Analysis via ICP-AES

1. Calibrate the ICP-AE spectrophotometer using multiple element standards following manufacturer's recommendations for the operation and calibration of the instrument.
2. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

## Calculations

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the Mehlich-1 extract to various concentration units in soil on a volume (mg $dm^{-3}$), weight (mg $kg^{-1}$), or area basis (lbs $acre^{-1}$).

   a. Volume basis
   Mehlich (1953) developed the Mehlich-1 method using a scooped volume of processed soil to report nutrient concentration on a volume basis as shown below.

   Volume basis, mg $dm^{-3}$ =
   mg $L^{-1}$ × (0.020 L Mehlich-1 ÷ 4 $cm^3$ soil) × (1000 $cm^3$ ÷ 1 $dm^3$) = mg $L^{-1}$ × 5

   b. Weight basis
   The average density of processed coarse-textured soil is 1.25 g $cm^{-3}$ which results in 4 $cm^3$ of soil being equal to 5 g. For 5 g of soil, the following formula is used to determine mg $kg^{-1}$.

   Weight basis, mg $kg^{-1}$ =
   mg $L^{-1}$ × (0.020 L Mehlich-1 ÷ 5 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 4

   c. Area basis
   Nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula with a 6 inch sampling depth and the assumption that soil weight in an acre at 6 inch depth is 2 million pounds.

   Area basis, lbs $acre^{-1}$ =
   mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.020 L Mehlich-1 ÷ 5 g) ×
   (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 8

## Analytical Performance

Range and Sensitivity

1. Instrument sensitivities allow reporting P, K, Ca, Mg, and Na to the nearest 1 mg $kg^{-1}$ and Fe, Mn, Cu, B, and Zn to the nearest 0.1 mg $kg^{-1}$.
2. For P, K, Ca, Mg, and Na, detection limits are much lower than concentrations observed in the extracts. For Fe, Mn, Cu, B, and Zn, detection limits may be close to the concentrations in the extracts and may need quantified for ensuring accurate results.
3. Analytical results are only valid to the concentration of the highest calibration standard. If concentrations exceed these values, the sample should be diluted and reanalyzed.

Alternatively, another calibration standard can be prepared at a concentration higher than the highest standard if the standard curve remains linear or is not extremely curvilinear.

Precision and Accuracy

1. Typical measurements of intralaboratory precision are shown in Table 2.

*Table 2. Intralaboratory precision for P and K in Mehlich-1 extracts. Average means and standard deviations are reported for three replications on samples from the Agricultural Laboratory Proficiency (ALP) program (ALP, 2013) .*

| Sample | P mean | P standard deviation | K mean | K standard deviation |
|---|---|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | | | |
| SRS - 0913 | 57 | 0.8 | 114 | 1.2 |
| SRS - 0914 | 26 | 0.7 | 50 | 1.5 |
| SRS - 0915 | 5 | 0.5 | 23 | 0.4 |

## Interpretation

1. Mehlich-1 is primarily used in states dominated by coastal plain soil. Correlation and calibration of the test results to fertilizer recommendations have been developed by land-grant Universities for several crops grown within states of the Southeastern US (Chapter 1.2). A summary of data used to develop fertilizer recommendations for peanuts and cotton on coastal plain soils can be found in Mitchell (1994) and Mitchell (2010). General soil fertility indices for Mehlich-1 P, K, Mg, and Ca are shown in Table 3. The actual ranges and corresponding fertilizer recommendations vary according to the soil, climate, and crop to be grown. For specific fertilizer recommendations, consult University extension publications in states where Mehlich-1 is used.

*Table 3. General soil fertility indices for Mehlich-1 P, K, Mg, and Ca.*

| Index | P | K | Mg | Ca |
|---|---|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | | | |
| Low | <10 | <35 | | |
| Medium | 10 to 20 | 35 to 80 | <30 | <500 |
| High | >20 | >80 | >30 | >500 |

2. Cation exchange capacity (CEC) of soil has an important role in determining sufficiency ranges and agronomic critical values for P and K. For Mehlich-1 P, the critical value for P decreases as CEC increases. For Mehlich-1 K, the critical value for K increases as CEC increases. Alabama estimates CEC from soil test results (see Chapter 6.2) while Georgia and South Carolina estimate CEC from physiographic regions soils were sampled from (Chapter

1.2). Phosphorus critical values decrease as CEC increases due to neutralization of acidity in the Mehlich-1 extractant in higher CEC soils or resorption of bioavailable P dissolved by Mehlich-1 onto clays and oxides (Kamprath and Watson, 1980). Potassium critical values increase with greater CEC due to greater retention of K by clay minerals.

3. Mehlich-1 was developed primarily to evaluate plant-available P, K, Mg, and Ca (Mehlich, 1953). With the advent of ICP-AES, the analysis of many more plant nutrients in the extract became possible. However, there is limited correlation and calibration research that can be used to develop fertilizer recommendations from soil-test results. University laboratories mostly recommend a single application rate of a micronutrient for specific crop or soil conditions known to have the potential for micronutrient deficiencies regardless of Mehlich-1 results.
4. Correlation and calibration studies for creating fertilizer P recommendation were developed with molybdate-blue spectrophotometric analysis of P rather than ICP-AE analysis. In Mehlich-3 extracts, phosphorus concentrations can be approximately 30% to 50% greater using ICP-AE compared to molybdate-blue spectrophotometry (Pittman et al., 2005). It is not known if a similar difference occurs in Mehlich-1 extracts.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the Mehlich-1 P measurement.
2. The Mehlich-1 extraction solution is stable and can be stored for several weeks due to its acidic nature. A specific shelf life is not known.
3. Reagent A in the molybdate-blue spectrophotometric method for P will last at least four months. Reagent B in the same method has a very short shelf-life and needs to be prepared daily.

## Safety and disposal

1. The chemicals used in this procedure pose no safety risk with safe handling procedures. Chemicals should be stored and disposed of according to routine laboratory procedures.
2. Some labs may require the acidity of the Mehlich-1 extracts be neutralized before discarding into the sink.
3. It is advisable to remove the bulk of soil particles from the waste stream before discharging.

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*Chapter 4.3*

# Mehlich-3

*H. Zhang, D.H. Hardy, R. Mylavarapu, and J.J. Wang*

## Application and Principle

The Mehlich-3 method estimates availability of most plant nutrients in soil using a solution with salt, dilute acid, fluoride, and EDTA buffered with acetic acid at pH 2.5. The method was developed by Mehlich in North Carolina to improve the Mehlich-1 extractant (0.05 *M* HCl and 0.0125 *M* $H_2SO_4$) that did not show a good correlation between extracted phosphorus and crop growth for all the variable soil types across the state (Mehlich, 1984). Lower critical soil test levels for Mehlich-1 in fine-textured soil compared to heavy-textured soil (Kamprath and Watson, 1980) indicated Mehlich-1 was not dissolving a portion of bioavailable P on fine-textured soil that was associated with aluminum. Fluoride was added in the Mehlich-3 extractant to complex aluminum in solution which acted to dissolve aluminum phosphates and prevent refixation of dissolved P (Nelson et al., 1953). Fluoride can precipitate as fluorite ($CaF_2$) on the surface of calcium carbonate in soil and the resultant precipitate has a high affinity for resorbing dissolved P in the extract at higher pH (Gunawan et al., 2010; Smillie and Syers, 1972; Turner et al., 2005; Turner et al., 2010). To prevent P resorption, acetic acid was added to the Mehlich-3 extractant to serve as a pH buffer to keep pH at 2.5 (Mehlich, 1978a; Mehlich, 1978b). Ammonium nitrate salt in Mehlich-3 serves to extract exchangeable cation nutrients such as calcium (Ca), magnesium (Mg), and potassium (K). The chelate, EDTA, was added to improve the extraction of micronutrients from soil with a particular emphasis on copper, manganese, and zinc (Mehlich, 1984). There was a Mehlich-2 extractant that was developed in between Mehlich-1 and Mehlich-3 that is not currently used. The Mehlich-2 extractant contained salt, dilute acid, and fluoride buffered with acetate at pH 2.5 (Mehlich, 1978b). The salt and acids were chlorides and EDTA was not present. The chloride and acid had undesirable corrosive properties in the lab. Changing the salts to nitrate and adding EDTA resulted in the Mehlich-3 extractant (Mehlich, 1984).

Mehlich had a philosophy of developing an extractant that could measure a wide range of plant nutrients to improve efficiency in soil testing. The Mehlich-3 method he developed is a versatile method that has been widely adopted by many laboratories across the US and the world. Although the method was developed to work well for P on soils with acid to neutral pH, the method has also proven to work well for P on calcareous soils due to the pH buffering potential of acetic acid (Lucero et al., 1998; Mallarino, 1997; Tran et al., 1990; Wang et al., 2004). As with any soil test method, interpretation of test results for making nutrient application recommendations will have some variation from region to region due to differences in soils, climates and crops. Mehlich-3 extractable nutrients have been calibrated to nutrient recommendation rates to maximize crop growth in several states in the Southeastern US to encompass these differences.

## Equipment and Apparatus

1. Soil scoop with 2.5 $cm^3$ capacity if measuring soil by volume
2. Analytical balance with 0.01 g resolution or 2 g calibrated scoop if measuring soil by weight

3. Analytical balance with 0.01 g resolution for making Mehlich-3 extractant
4. Polyethylene carboy with 20 L capacity
5. Reciprocating mechanical shaker capable of 180 oscillations per minute
6. Volume dispenser for delivering 25 mL Mehlich-3 extractant
7. Volume dispenser for delivering 27 mL of reagent for spectrophotometric P analysis.
8. Extraction bottles or flasks (50 to 100 mL) with stoppers
9. Filter funnels and vials for receiving filtrates
10. Whatman No. 1 filter paper or equivalent
11. Vortex stirrer if analyzing P via molybdate-blue spectrophotometry
12. Spectrophotometer, automated segmented flow analyzer, or flow injection analyzer with capability to measure absorbance at 882 nm if analyzing P via molybdate-blue spectrophotometry
13. Inductively coupled plasma-atomic emission (ICP-AE) spectrometer.

## Reagents

1. *Mehlich-3 extractant*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 0.2 *M* acetic acid, 0.25 *M* ammonium nitrate, 0.015 *M* ammonium fluoride, 0.013 *M* nitric acid, and 0.001 *M* ethylenediaminetetraacetic acid.
   <u>Stock solution with 3.75 *M* $NH_4F$ and 0.25 *M* EDTA</u>
   a. In a 2 L volumetric flask, add about 1200 mL $H_2O$
   b. Add 277.8 g ammonium fluoride ($NH_4F$) and thoroughly stir to dissolve.
   c. Add 146.1 g of ethylenediaminetetraacetic acid (EDTA, $((HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2)$ and thoroughly stir to dissolve.
   d. Bring solution to 2 L volume with deionized water and thoroughly stir.
   e. Store stock solution in plastic bottle.
   <u>Mehlich-3</u>
   f. Fill a 20-L calibrated plastic carboy with approximately 12 L of deionized water.
   g. Add 400 g of ammonium nitrate ($NH_4NO_3$) and thoroughly stir to dissolve.
   h. Add 80 mL of stock solution containing with 3.75 *M* $NH_4F$ and 0.25 *M* EDTA. Thoroughly stir to dissolve.
   i. Add 230 mL concentrated glacial acetic acid ($CH_3COOH$) and 16.4 mL concentrated $HNO_3$. Thoroughly stir.
   j. Bring solution to 20-L with deionized water and thoroughly stir.
   k. The pH of the extracting solution is approximately 2.5.
2. *Reagents for molybdate-blue spectrophotometric analysis of P:* The reagents described here were taken from Tucker (1992). The reagents were developed from Watanabe and Olsen (1965) who modified the original method by Murphy and Riley (1962) with a reagent made on a daily basis. Reagent A has more concentrated components than the comparable reagent from Watanabe and Olsen (1965) since the reagent is diluted when creating Reagent C.
   <u>*Reagent A (sulfuric-molybdate-tartrate solution)*</u>
   a. In a 2 L volumetric flask, dissolve 100 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 500 mL of deionized water.
   b. Dissolve 2.425 g of antimony potassium tartrate ($K(SbO)\cdot C_4H_4O_6\cdot ½\ H_2O$) in the molybdate solution just prepared.

c. Slowly add 1400 mL of concentrated $H_2SO_4$ (18 *M*). Stir to ensure thorough mixing. The solution will heat up upon adding $H_2SO_4$.
d. Let the solution cool to room temperature then dilute to 2 L with deionized water. Store in an opaque polyethylene or Pyrex glass bottle in a dark and refrigerated environment.

*Reagent B (ascorbic acid solution)*

a. In a 2 L volumetric flask, dissolve 176 g ascorbic acid ($C_6H_8O_6$) and dilute to 2 L. Store in an opaque polyethylene or Pyrex glass bottle in a dark and refrigerated environment.

*Reagent C (working solution)*

a. Add 20 mL of Reagent A and 10 mL of Reagent B to a 1 L volumetric flask and dilute to volume with deionized water. Allow solution to come to room temperature before using. Prepare this reagent fresh daily.

3. *Calibrations Standards for analysis via ICP-AE spectrometry (ICP-AES)*
   a. From commercially purchased standard solutions containing 1,000 mg $L^{-1}$ of each analyte, prepare 1 L of a standard in Mehlich-3 extracting solution containing the highest concentration of each element. This is a calibration standard with the highest concentration of analytes.
   b. Prepare additional calibration standards by diluting the most concentrated calibration standard with Mehlich-3. A recommended concentration range for calibration standards is shown in Table 1 for ICP-AES. If analyzing P with molybdate-blue spectrophotometry, prepare a blank and standards with 1, 2, 5, 10, 15, and 20 mg $L^{-1}$ P.
   c. Cations (K, Ca, Mg, Na, Fe, Mn, Cu, Zn) can be grouped together in the same calibration standards for ICP-AES. Likewise, anion (P, B) analytes can be grouped together. Avoid combining cation and anion analytes in the same standards since precipitation may remove analytes from solution.

*Table 1. Suggested calibration standard concentrations (mg $L^{-1}$) for quantitative analysis of Mehlich-3 soil extracts using ICP-AES.*

| | P | K | Ca | Mg | Na | Fe | Mn | Cu | Zn | B |
|---|---|---|---|---|---|---|---|---|---|---|
| Blank | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Standard 1 | 10 | 10 | 50 | 10 | 10 | 10 | 2 | 2 | 2 | 2 |
| Standard 2 | 25 | 25 | 100 | 50 | 25 | 25 | 5 | 5 | 5 | 5 |
| Standard 3 | 50 | 50 | 500 | 100 | 50 | 50 | 10 | 10 | 10 | 10 |

## Procedure

Extraction

1. Scoop 2.5 $cm^3$ or measure 2 g of processed soil (dried, < 2 mm) and add to an extraction flask.
2. Add 25 mL of Mehlich-3 extracting solution to the flask.
3. Twenty-five mL of Mehlich-3 and 2.5 $cm^3$ of soil were used in the originally developed method (Mehlich, 1984). These quantities result in a 10:1 extractant/soil volume ratio. As the Mehlich-3 extract became adopted by other laboratories, a ratio of 10:1 was used with soil measured on a weight basis. For example, 20 mL of Mehlich-3 and 2 g of soil are

commonly used quantities. The weight of soil is either measured with a balance or approximated with a 1.7 $cm^3$ scoop volume which assumes density of processed soil is 1.18 g $cm^{-3}$ (Peck, 1998). Whether using a 10:1 ratio based on soil volume or weight, fertilizer recommendations from the test results should be based on calibrations that used soil test results with the same ratio.

4. Add stoppers to flasks and shake soil and Mehlich-3 for 5 min on a reciprocating mechanical shaker with a minimum of 180 oscillations per minute.
5. Filter the suspension using Whatman No. 1 filter paper and collect the filtrate in vials for analysis. Refilter if filtrate is cloudy.

Phosphate Analysis via Molybdate-Blue Spectrophotometry

1. Dilute 1 mL of sample extract or calibration standard with 27 mL of Reagent C and stir well.
2. Allow color to develop for at least 20 minutes.
3. Adjust and operate the spectrophotometer in accordance with manufacturer's instructions. Read absorbance at a wavelength of 880 nm. Adjust the absorbance of the calibration blank with 0 mg $L^{-1}$ P to read an absorbance of 0. Determine absorbance of calibration standards and unknown extracts. Calculate P concentrations of unknown extracts from a standard curve of absorbance versus P concentrations of calibration standards. If the absorbance of an unknown extract exceeds the absorbance of the highest calibration standard, the extract should be diluted with Mehlich-3 so absorbance of the unknown is within the absorbance range of the standard curve.
4. If using automated spectrophotometric analysis such as flow-injected analysis or segmented flow, follow instrument manufacturer's instructions for P analysis.

Analysis via ICP-AES

1. Calibrate the ICP-AE spectrophotometer using multiple element standards following manufacturer's recommendations for the operation and calibration of the instrument.
2. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

## Calculations

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the Mehlich-3 extract to various concentration units in soil on a volume (mg $dm^{-3}$), weight (mg $kg^{-1}$), or area basis (lbs $acre^{-1}$).

    a. Volume basis
    Mehlich (1984) developed the Mehlich-3 method using a scooped volume of processed soil to report nutrient concentration on a volume basis as shown below.

    Volume basis, mg $dm^{-3}$ =
    mg $L^{-1}$ × (0.025 L Mehlich-3 ÷ 2.5 $cm^3$ soil) × (1000 $cm^3$ ÷ 1 $dm^3$) = mg $L^{-1}$ × 10

    If the density of processed soil is assumed to be 1 g $cm^{-3}$, mg $dm^{-3}$ is equivalent to mg $kg^{-1}$.

b. Weight basis

An alternative method of measuring soil by weight has been adopted by most laboratories. With measurement of 2 g soil extracted with 20 mL Mehlich-3, the concentration in soil as mg $kg^{-1}$ is determined by the following formula.

Weight basis, mg $kg^{-1}$ =

mg $L^{-1}$ × (0.020 L Mehlich-3 ÷ 2 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 10

The weight of soil is either measured directly or approximated with a 1.7 $cm^3$ scoop that assumes density of processed soil is 1.18 g $cm^{-3}$. This is an average density for silt loam soils.

c. Area basis

When measuring soil by weight, nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula with a 6 inch sampling depth and the assumption that soil weight in an acre at 6 inch depth is 2 million pounds.

Area basis, lbs $acre^{-1}$ =

mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.020 L Mehlich-3 ÷ 2 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 20

If measuring soil by volume with a Mehlich-3 to soil ratio of 10:1, the same multiplication factor of 20 can be used if the processed soil density is assumed to be 1 g $cm^{-3}$.

## Analytical Performance

Range and Sensitivity

1. Instrument sensitivities allow reporting P, K, Ca, Mg, and Na to the nearest 1 mg $kg^{-1}$ and Fe, Mn, Cu, B, and Zn to the nearest 0.1 mg $kg^{-1}$.
2. For P, K, Ca, Mg, and Na, detection limits are much lower than concentrations observed in the extracts. For Fe, Mn, Cu, B, and Zn, detection limits may be close to the concentrations in the extracts and may need quantified for ensuring accurate results.
3. Analytical results are only valid to the concentration of the highest calibration standard. If concentrations exceed these values, the sample should be diluted and reanalyzed. Alternatively, another calibration standard can be prepared at a concentration higher than the highest standard if the standard curve remains linear or is not extremely curvilinear.

Precision and Accuracy

1. Repeatability (intralaboratory precision) of Mehlich-3 extracted plant nutrients was evaluated in 26 laboratories (Zhang et al., 2009; Schroder et al., 2009). A summary of the findings are shown in Table 2 as % relative standard deviation (% RSD).

*Table 2. Ranges of intralaboratory precision for P, K, Ca, Mg, Zn, Mn, Fe, and Cu in Mehlich-3 extracts. P(blue) was P determined by molybdate-blue spectrophotometry. All others were determined by ICP-AES. Precision ranges shown as % relative standard deviation (% RSD) of three replicates for 10 soils sent to 26 labs for P and 11 soils sent to 23 labs for the other nutrients.*

| Nutrient | Soil measured with scoop | Soil measured by weight |
|---|---|---|
| | ---------- % RSD ---------- | |
| P(blue) | 2.1 to 12.1 | 1.1 to 9.3 |
| P | 2.2 to 21.4 | 1.7 to 5.8 |
| K | 3.9 to 6.1 | 1.6 to 4.6 |
| Ca | 2.2 to 10.6 | 1.4 to 16.9 |
| Mg | 2.4 to 5.7 | 1.4 to 9.8 |
| Zn | 4.1 to 42.7 | 2.6 to 33.0 |
| Mn | 3.2 to 8.5 | 1.6 to 4.9 |
| Fe | 2.3 to 7.7 | 1.6 to 4.5 |
| Cu | 3.6 to 11.2 | 3.0 to 17.9 |

2. Reproducibility (interlaboratory precision) of Mehlich-3 extracted plant nutrients were also determined by Zhang et al. (2009) and Schroder et al. (2009). A summary of the findings are shown in Table 3 as % RSD.

*Table 3. Ranges of interlaboratory precision for P, K, Ca, Mg, Zn, Mn, Fe, and Cu in Mehlich-3 extracts. P(blue) was P determined by molybdate-blue spectrophotometry. All others were determined by ICP-AES. Precision ranges shown as % relative standard deviation (% RSD) of values obtained for 10 soils across 26 labs for P and 11 soils across 23 labs for the other nutrients.*

| Nutrient | Soil measured with scoop | Soil measured by weight |
|---|---|---|
| | ---------- % RSD ---------- | |
| P(blue) | 1.6 to 50.8 | 7.2 to 42.6 |
| P | 7.0 to 74.0 | 5.3 to 35.9 |
| K | 7.4 to 20.0 | 3.5 to 12.7 |
| Ca | 7.1 to 33.5 | 7.6 to 34.6 |
| Mg | 8.5 to 26.4 | 8.1 to 29.0 |
| Zn | 11.6 to 42.8 | 11.9 to 49.2 |
| Mn | 10.5 to 19.7 | 7.5 to 20.7 |
| Fe | 12.4 to 22.0 | 11.0 to 21.5 |
| Cu | 8.9 to 45.3 | 9.7 to 43.0 |

Interferences

1. Positive interferences can occur with the molybdate-blue spectrophotometric method in the presence of silicate or arsenate since they react with the molybdate reagent in a manner similar to phosphate. These interferences are not expected to be significant in Mehlich-3 extracts. Silicate interference occurred at P concentrations common in soil solution which are much lower than those in Mehlich-3 extracts (Ciavatta et al., 1990). Although arsenate has a sensitivity similar to P (Tsang et al., 2007), concentrations of arsenate are expected to be much lower than P in Mehlich-3 extracts.
2. The molybdate-blue spectrophotometric method was developed to only detect orthophosphate. Positive interferences may occur with hydrolysis of organic P compounds to orthophosphate in the acid extract. An additional reagent can be added to the method to remove excess molybdate reacting with orthophosphate from hydrolyzed organic P compounds (Dick and Tabatabai, 1977).
3. Time and temperature in which absorbance measurements are taken for calibration standards and Mehlich-3 extracts should be consistent since development of the blue molybdate-phosphate complex is sensitive to these parameters (Towns, 1986).
4. Phosphorus analyzed via ICP-AES can be 30 to 50% greater than P analyzed via molybdate-blue spectrophotometry (Mallarino, 2003; Pittman et al., 2005; Ziadi et al., 2009). Very minor difference between the two methods was observed elsewhere (Sikora et al., 2005). Although there are several hypotheses, no clear evidence is available to explain why the difference between ICP-AES and molybdate-blue spectrophotometry occurs (Pittman et al., 2005; Ziadi et al., 2009).
5. Boron analysis in Mehlich-3 extracts is problematic. Difficulty arises in maintaining a stable baseline since B concentration of a blank solution consistently drifts upwards. Fluoride in Mehlich-3 may release B from borosilicate glass. Or, B may be absorbed by glass and desorbed at a later time. If B is determined, all solutions should be stored in plastic and plastic funnels should be used. Even with use of plasticware, B analysis with ICP-AES is prone to difficulty due to the glass nebulizer and torch in contact with the solution before entering the plasma.

## Interpretation

1. Mehlich-3 is a versatile extractant that has been shown to work well across a wide range of soils from acid to alkaline nature. Correlation and calibration of the test results to fertilizer recommendations have been developed by land-grant Universities for individual states of the Southeastern US (Chapter 1.2). General soil fertility indices for Mehlich-3 P, K, Mg, and Ca values are shown in Table 4. The actual ranges and corresponding fertilizer recommendations vary according to the soil, climate, and crop to be grown. For specific fertilizer recommendations, consult University extension publications in states where Mehlich-3 is used.
2. Cation exchange capacity (CEC) of soil has an important role in determining sufficiency ranges and critical values for K. The critical value for Mehlich-3 K increases as CEC increases. Potassium critical values increase with greater CEC due to greater retention of K by clay minerals competing with root uptake. Louisiana State University provides K recommendations based on soil CEC since Louisiana has soils with a wide range of CEC from sandy loams with 4 cmol $kg^{-1}$ to clays with >20 cmol $kg^{-1}$ (Chapter 1.2).

*Table 4. General soil fertility indices for Mehlich-3 P, K, Mg, and Ca (mg $kg^{-1}$).*

| Index | P | K | Mg | Ca |
|---|---|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | | | |
| Low | <20 | <90 | | |
| Medium | 20 to 35 | 90 to 130 | <40 | <1000 |
| High | >35 | >130 | >40 | >1000 |

3. Mehlich-3 was primarily developed to evaluate plant-available P, K, Mg, Ca, Mn, Zn, and Cu (Mehlich, 1984). Phosphorus was determined via molybdate-blue spectrophotometry, K was determined by flame emission spectrophotometry, and the rest of the nutrients determined by atomic absorption spectrophotometry. With the advent of ICP-AES, the efficiency of analysis was greatly improved since all the nutrients could be analyzed simultaneously. Also, other nutrients could be analyzed in the extract such as Na, Fe, and B. Although ICP-AES can analyze a wide range of nutrients in the Mehlich-3 extract, there is limited correlation and calibration research that can be used to develop fertilizer recommendations from soil-test results. University laboratories mostly recommend a single application rate of a micronutrient for specific crop or soil conditions known to have the potential for micronutrient deficiencies regardless of Mehlich-3 results.
4. Correlation and calibration studies for creating fertilizer P recommendations were developed with molybdate-blue spectrophotometric analysis of P rather than ICP-AE analysis. Phosphorus concentrations have been observed to be 30 to 50% greater with ICP-AES compared to molybdate-blue spectrophotometry (Mallarino, 2003; Pittman et al., 2005; Ziadi et al., 2009). In Iowa, a field correlation study was performed to develop distinct agronomic critical values and soil fertility indices for corn with Mehlich-3 P determined via ICP-AES (Mallarino, 2003). In Oklahoma, a regression equation between Mehlich-3 P analyzed via ICP-AES and molybdate-blue spectrophotometry was developed to calculate a molybdate-blue spectrophotometric P value from ICP-AE analysis of P (Pittman et al., 2005).

## Effects of Storage

1. Air-dried soils may be stored several months without affecting results.
2. The Mehlich-3 extraction solution is stable and can be stored for several weeks due to its acidic nature. A specific shelf life is not known.
3. Reagent A in the molybdate-blue spectrophotometric method for P will last at least four months. Reagent B in the same method has a very short shelf-life and needs to be prepared daily.

## Safety and disposal

1. The chemicals used in this procedure pose no safety risk with safe handling procedures. Chemicals should be stored and disposed of according to routine laboratory procedures.
2. Some labs may require the acidity of the Mehlich-3 extracts be neutralized before discarding into the sink.
3. It is advisable to remove the bulk of soil particles from the waste stream before discarding.

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*Chapter 4.4*

# Lancaster

*J.L. Oldham*

## Application and Principle

The Lancaster soil test method was developed to test the diversity of soils in Mississippi that can be either acidic or alkaline in nature (Lancaster, 1980). The method is also commonly referred to as the Mississippi soil test method. The chemistry of the extractant used in the method was developed with a primary focus on determining plant-available phosphorus (P) but it has also been effective in determining plant-available potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), and zinc (Zn).

Development of the Lancaster method was based on the principle that the important soil phosphorus fractions that become available to plants follows the order aluminum-P > calcium-P > iron-P (Lancaster, 1980). Two stages are performed in the extraction (Crouse, 2001). In the first stage, a weak acidic solution is reacted with soil without agitation to result in dissolution of plant-available calcium-P while keeping plant-unavailable apatitic calcium phosphate minerals undissolved. The second stage involves extraction of aluminum-P with addition of a solution containing fluoride and organic acids that form soluble complexes with aluminum which promotes aluminum-P dissolution. Fluoride is present at low concentration to minimize formation of Ca and Mg fluoride precipitates which would remove them from solution and prevent them from being extracted from soil. Aluminum is present in the extract to ensure formation of soluble aluminum-fluoride complexes which further minimizes Ca and Mg fluoride precipitation. The organic acids in the second stage extract are acetic, malic, and malonic which serve to buffer the pH at 4.0.

Various comparisons have been made between results from the Lancaster method and other soil test methods. For soils with pH less than 7, Rasberry and Lancaster (1977) found Ca, Mg, and Mn extracted in the Lancaster method was very similar to the elements extracted in the 1 M ammonium acetate method. On both acid and alkaline soils in Mississippi, Jittanoonta (1980) found the Lancaster and Mehlich-2 methods to both produce soil test P values with good correlation to P uptake of pearl millet in a greenhouse study while Mehlich-1 provided a poor correlation. The Lancaster method produced extractable K, Ca, and Mg values that were correlated well to Mehlich-3 K, Ca, and Mg (Cox, 2011). However, a poor correlation was observed between Lancaster P and Mehlich-3 P.

## Equipment and Apparatus

1. Analytical balance with 0.01 g resolution for measuring soil
2. Analytical balance with 0.1 g resolution for making Lancaster reagents
3. Polyethylene carboy with 20 L capacity
4. Reciprocating mechanical shaker capable of 180 oscillations per minute
5. Volume dispenser for delivering 5 and 20 mL of extractant solutions
6. Erlenmeyer flasks (50 mL) for soil extraction

7. Filter funnels and vials for receiving filtrates
8. Whatman No. 2 filter paper, 11 cm (or equivalent)
9. Powder funnels for adding soil to Erlenmeyer flasks
10. Inductively coupled plasma - atomic emission (ICP-AE) spectrophotometer

## Reagents

1. *Solution A for first stage of extraction*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 0.05 *M* HCl.
   a. Add approximately 15 L of deionized water to a calibrated 20 L carboy.
   b. Add 83.3 mL of concentrated HCl
   c. Add water to the 20 L mark and stir thoroughly.
2. *Solution B for second stage of extraction*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 1.57 *M* glacial acetic acid, 0.063 *M* malonic acid, 0.089 *M* malic acid, 0.032 *M* ammonium fluoride, 0.012 *M* aluminum chloride hexahydrate.
   a. Add about 8 L of deionized water to a calibrated 20 L carboy.
   b. Add 1800 mL concentrated glacial acetic acid ($CH_3COOH$) and thoroughly stir.
   c. Add 130.4 g malonic acid ($CH_2(COOH)_2$) and thoroughly stir to dissolve.
   d. Add 240 g malic acid ($HO_2CCH_2CH(OH)CO_2H$) and thoroughly stir to dissolve.
   e. Add 24 g ammonium fluoride ($NH_4F$) and thoroughly stir to dissolve.
   f. Add 60 g aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) and thoroughly stir to dissolve.
   g. Add 500 mL concentrated ammonium hydroxide ($NH_4OH$, 28-30% $NH_3$) and thoroughly stir to dissolve.
   h. Dilute to 20 L with deionized water and thoroughly stir. The pH of this solution should be 4.0.
3. *Solution C for calibration blank and standards*
   a. Add 200 mL of Solution A to a 1 L volumetric flask.
   b. Dilute to 1 L with solution B and thoroughly mix.
   c. Use this solution for preparing calibration blanks and standards.
4. *Calibrations Standards*
   a. From commercially purchased standard solutions containing 1,000 mg $L^{-1}$ of each analyte, prepare 1 L of a standard in solution C containing the highest concentration of each element. This is a calibration standard with the highest concentration of analytes.
   b. Prepare additional calibration standards by diluting the most concentrated calibration standard with solution C. A recommended concentration range for calibration standards is shown in Table 1.
   c. Cation (K, Ca, Mg, Zn) analytes can be grouped together in the same calibration standards for ICP-AES. Avoid combining cations with P in the same solution since precipitates may form removing analytes from solution.

*Table 1. Suggested calibration standard concentrations (mg $L^{-1}$) for quantitative analysis of Lancaster soil extracts using ICP-AES.*

| | P | K | Ca | Mg | Zn |
|---|---|---|---|---|---|
| Blank | 0 | 0 | 0 | 0 | 0 |
| Standard 1 | 5 | 5 | 40 | 20 | 0.1 |
| Standard 2 | 10 | 20 | 400 | 60 | 0.5 |
| Standard 3 | 40 | 60 | 800 | 200 | 2.0 |

## Procedure

Extraction

1. Measure 5 g of processed soil (dried, < 2 mm) and add to 50-mL Erlenmeyer flask using a powder funnel.
2. Add 5 mL of Solution A.
3. Keep sample stationary for 10 min.
4. Add 20 mL of Solution B.
5. Shake on reciprocating shaker for 10 min at a minimum of 180 oscillations per minute.
6. Filter suspension through Whatman No. 2 filter paper in a well-ventilated area and collect filtrate in vials for analysis.

Phosphate Analysis via Molybdate-Blue Spectrophotometry

1. University laboratories in the Southeastern US using the Lancaster method analyze P in the extract with inductively coupled plasma – atomic emission spectroscopy (ICP-AES). The Lancaster method was originally developed with P analysis via molybdate-blue spectrophotometry which can be found in Chapter 4.3.

Analysis via ICP-AES

1. Calibrate the ICP-AE spectrophotometer using multiple element standards following manufacturer's recommendations for the operation and calibration of the instrument.
2. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

## Calculations

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the Lancaster extract to various concentration units in soil on weight (mg $kg^{-1}$) or area basis (lbs $acre^{-1}$).

    a. Weight basis
    Weight basis, mg $kg^{-1}$ =
    mg $L^{-1}$ × (0.025 L extractant ÷ 5 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 5

    b. Area basis
    Nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula with a 6 inch sampling depth and the assumption that soil weight in an acre at 6 inch depth is 2 million pounds.

Area basis, lbs $acre^{-1}$ =

mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.025 L extractant ÷ 5 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2 \times 10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 10

## Analytical Performance

Range and Sensitivity

1. Analytical results are only valid to the concentration of the highest calibration standard. If concentrations exceed these values, the sample should be diluted and reanalyzed. Alternatively, another calibration standard can be prepared at a concentration higher than the highest standard if the standard curve remains linear or is not extremely curvilinear.

Precision and Accuracy

1. Repeated analysis of eight standard samples over one month resulted in coefficients of variation from 5 to 10 %. This variance was attributed to soil variability rather than the procedure.

## Interpretation

1. Phosphorus and potassium recommendations from the Lancaster soil test method provided by the Mississippi State University Extension Service were developed through extensive field and greenhouse procedures comparing extracted nutrients to crop response indices. Soil test indices for P and K are shown in Tables 1 and 2. Potassium recommendations are based on both the extracted K and the estimated cation exchange capacity of the soil.

*Table 1. Soil fertility indices for P with the Lancaster extractant for all crops.*

| Index | Soil Test P mg $kg^{-1}$ |
|---|---|
| Very Low | < 10 |
| Low | 10 - 18 |
| Medium | 19 - 36 |
| High | 37 - 72 |
| Very High | > 72 |

*Table 2. Soil fertility indices for K with the Lancaster extractant for various crops at different cation exchange capacity (CEC) ranges.*

| Index | CEC <7 | CEC 7 - 14 | CEC 15 - 25 | CEC >25 |
|---|---|---|---|---|
| | ---------- cmol $kg^{-1}$ ---------- | | | |
| | Soil Test K, mg $kg^{-1}$ (for Group A) | | | |
| Very Low | < 21 | < 26 | < 31 | < 36 |
| Low | 21 - 40 | 26 - 55 | 31 - 65 | 36 - 75 |
| Medium | 41 - 60 | 56 - 80 | 66 - 90 | 76 - 100 |
| High | 61 - 105 | 81 - 140 | 91 - 157 | 101 - 175 |
| Very High | > 105 | > 140 | > 157 | > 175 |
| | Soil Test K, mg $kg^{-1}$ (for Group B) | | | |
| Very Low | < 26 | < 31 | < 36 | < 41 |
| Low | 26 - 55 | 31 - 70 | 36 - 80 | 41 - 90 |
| Medium | 56 - 80 | 71 - 95 | 81 - 105 | 91 - 120 |
| High | 81 - 140 | 96 - 167 | 106 - 185 | 121 - 210 |
| Very High | > 140 | > 167 | > 185 | > 210 |
| | Soil Test K, mg $kg^{-1}$ (for Group C) | | | |
| Very Low | < 36 | < 46 | < 60 | < 75 |
| Low | 36 - 75 | 46 - 95 | 61 - 120 | 76 - 130 |
| Medium | 76 - 100 | 96 - 120 | 121 - 145 | 131 - 160 |
| High | 101 - 175 | 121 - 210 | 146 - 255 | 161 - 280 |
| Very High | > 175 | > 210 | > 255 | > 280 |

Group List A: perennial winter grass pasture (fescue or orchard grass), small grains for pasture, peanuts, perennial summer grass pasture (bahia, dallis, or Bermuda), rice, or annual legumes with ryegrass
Group List B: dryland corn for grain, soybeans, oats, wheat, barley, summer pastures (bahia, dallis, or Bermuda) with annual legumes (white clover, red clover, lespedeza, arrowleaf clover, ball clover, or subterrean clover), temporary summer grass pastures (millet, sorghum, sudangrass, sorghum-sudangrass hybrids, or johnsongrass), forage legumes, perennial winter grass pasture with clover (white clover, red clover, subterranean clover with fescue or orchardgrass), pasture grass with annual legumes (crimson clover, annual lespedeza, arrowleaf clover, ball clover, or subterrean clover with bermuda, dallis, or bahia grass), Johnsongrass hay, mixed grass hay, annual or sericea Lespedeza hay, or sunflowers
Group List C: alfalfa, cotton, corn or sorghum for silage, sweet potatoes, irrigated corn, or hybrid Bermudagrass hay

2. Soil test indices for Mg are based on extracted Mg for CEC less than 5 cmol $kg^{-1}$ or percent Mg saturation of CEC for CEC greater than 5 cmol $kg^{-1}$ (Table 3). Magnesium deficiencies mostly occur on acidic soils. Magnesium applications via dolomitic limestone are recommended with soil test indices of very low, low, and medium.

*Table 3. Soil fertility indices for Mg with the Lancaster extractant for all crops.*

| Index | Soil Test Mg, mg $kg^{-1}$ CEC ≤ 5 | Mg saturation of CEC, % CEC > 5 |
|---|---|---|
| Very Low | < 6 | <0.85 |
| Low | 6 - 12 | 0.86 - 1.75 |
| Medium | 13 - 24 | 1.76 - 3.30 |
| High | 25 - 48 | 3.31 - 6.60 |
| Very High | > 48 | > 6.60 |

3. Corn is the only crop to show Zn deficiencies in Mississippi. Zinc deficiency rarely occurs at soil pH less than 6.0. The higher the pH, the greater the likelihood of Zn deficiency and a greater amount of soil test Zn is required. Table 4 provides soil test Zn indices for corn based on soil pH. Zinc applications are recommended for corn with very low, low, and medium soil test indices.

*Table 4. Soil fertility indices for Zn with the Lancaster extractant for corn.*

| | Soil Test Zn, mg $kg^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| Index | pH = 5.5 | pH = 6.0 | pH = 6.5 | pH = 7.0 | pH = 7.5 | pH = 8.0 |
| Very Low | < 0.15 | < 0.15 | < 0.20 | < 0.25 | < 0.30 | < 0.37 |
| Low | 0.16 - 0.30 | 0.18 - 0.35 | 0.21 - 0.40 | 0.26 - 0.50 | 0.31 - 0.60 | 0.38 - 0.75 |
| Medium | 0.31 - 0.60 | 0.36 - 0.70 | 0.41 - 0.80 | 0.51 - 1.00 | 0.61 - 1.20 | 0.76 - 1.50 |
| High | 0.61 - 1.80 | 0.71 - 2.10 | 0.81 - 2.40 | 1.01 - 3.00 | 1.21 - 3.60 | 1.51 - 4.50 |
| Very High | > 1.80 | > 2.10 | > 2.40 | > 3.00 | > 3.60 | > 4.50 |

## Effects of Storage

1. Solutions for extraction are stored in polyethylene carboys. They can be stored for an indefinite period of time without affecting soil test results.

## Safety and Disposal

1. The chemicals used in this procedure pose no safety risk with safe handling procedures. Chemicals should be stored and disposed of according to routine laboratory procedures.

## References

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*Chapter 4.5*

# DTPA and DTPA-Sorbitol Extraction of Micronutrients

*T. Provin and H. Zhang*

## Application and Principle

Extraction of soil with DTPA for micronutrients was developed by Lindsay and Norvell (1978) to overcome excessive levels being extracted with acid extracts on calcareous soils. Plant micronutrients extracted are iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). Although developed for calcareous soil, the method has proven useful for nearly all soil pH ranges. The advent of inductively coupled plasma-atomic emission spectrometry (ICP-AES) for simultaneous analysis of micronutrients in the extract provided improved efficiency compared to separate analyses of the micronutrients in the originally developed method using atomic absorption spectrometry.

Boron is another important micronutrient that was not included in the original DTPA extraction method. Plant-available boron (B) in soil is commonly extracted with refluxing boiling water (Berger and Truog, 1940). This method requires an extraction separate from DTPA. Vaughan and Howe (1994) reported on use of boron chelates to extract B rather than using the separate boiling water extraction method. Miller et al. (2000) documents the inclusion of sorbitol in the DTPA extractant for plant-available B. This DTPA-sorbitol method allows for simultaneous extraction of Fe, Zn, Cu, Mn, and B. Simultaneous analyses of the micronutrients in the extract then occurs with ICP-AES.

## Equipment and Apparatus

1. Calibrated 10 g scoop or balance with 0.1 g resolution for measuring soil
2. Analytical balance with 0.01 g resolution for making extractant
3. Polyethylene carboys with 20 L capacity
4. Volume Dispenser capable of dispensing 20 mL of extractant
5. Reciprocating shaker with a 2 to 2.5-inch movement at180 oscillations per minute or orbital shaker with a 1-inch throw at 180 revolutions per minute. Either shaker must be fitted with appropriate sample vessel holders.
6. 250 mL Erlenmeyer flask or similar extracting vessel
7. Filter funnels and vials for receiving filtrates
8. Whatman No. 2 filter paper
9. pH meter
10. ICP-AE spectrophotometer or atomic absorption spectrophotometer with appropriate lamps

## Reagents

1. *DTPA extractant*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution.

The solution contains 0.005 *M* diethylenetriaminepentaacetic acid, 0.01 *M* calcium chloride dihydrate, and 0.1 *M* triethanolamine with pH adjusted to 7.30.

a. Place a 1.5 inch stir bar into a polyethylene carboy calibrated with markings to volumes of 15 and 20 L.
b. Add deionized water into the carboy to the 15-L mark.
c. Weigh 39.89 g of diethylenetriaminepentaacetic acid (DTPA, $[(HOOCCH_2)_2NCH_2CH_2]_2 NCH_2COOH$) and add it to the carboy.
d. Weigh 29.44 g calcium chloride ($CaCl_2 \cdot 2H_2O$) and add to the carboy.
e. Measure 410 mL or 298 g of triethanolamine (TEA, $(HOCH_2CH_2)_3N$) and add it to the carboy.
f. Measure 85 mL of concentrated HCl and add it to the carboy.
g. Add deionized water to bring the volume to the 20 L mark.
h. Place carboy on a magnetic stirrer and stir overnight (minimum of 8 hr) at a speed of approximately 600 rpm.
i. Measure pH of the extractant. Adjust the pH to 7.3. Add 9 mL concentrated HCl for each pH unit over 7.3 with stirring. Add 9 mL TEA for each pH unit under 7.3 with stirring. Measure pH after 30 min of stirring and repeat the process until pH equals 7.3.

2. *DTPA-sorbitol extractant*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 0.005 *M* diethylenetriaminepentaacetic acid, 0.01 *M* calcium chloride dihydrate, 0.1 *M* triethanolamine, and 0.2 M sorbitol with pH adjusted to 7.30.
   a. Follow directions a through f for making DTPA extractant.
   b. Add 728.8 g sorbitol ($C_6H_{14}O_6$) to the carboy.
   c. Add deionized water to bring the volume to the 20 L mark.
   d. Place carboy on a magnetic stirrer and stir overnight (minimum of 8 hr) at a speed of approximately 600 rpm.
   e. Measure pH of the extractant. Adjust the pH to 7.3. Add 9 mL concentrated HCl for each pH unit over 7.3 with stirring. Add 9 mL TEA for each pH unit under 7.3 with stirring. Measure pH after 30 min of stirring and repeat the process until pH equals 7.3.
3. *Calibration standards*
   a. From commercially purchased standard solutions containing 1,000 mg $L^{-1}$ of each analyte, add appropriate amounts to 1 L volumetric flasks and dilute to volume to prepare 1 L of standard. Suggested concentrations of standards are shown in Table 1.
   b. A working standard of 100 mg $L^{-1}$ should be prepared to create calibration standards with analyte concentrations less than 1 mg $L^{-1}$.
   c. If B is included in the standards, use plastic volumetric flasks or immediately transfer solutions from glass volumetric flasks to plastic bottles to avoid B contamination from glassware.

## Procedure

Extraction

1. Scoop or weigh 10 g of processed soil (<2 mm) into 500-mL Erlenmeyer flasks. If the soil is measured with a scoop, assume a density of 1.18 g $cm^{-3}$ for fine-textured soils and use an 8.5 $cm^3$ scoop. For coarse-textured soils, assume a density of 1.25 g $cm^{-3}$ and use an 8 $cm^3$ scoop.

*Table 1. Suggested calibration standard concentrations (mg $L^{-1}$) for quantitative analysis of DTPA or DTPA-sorbitol soil extracts using ICP-AES.*

| | Fe | Zn | Mn | Cu | B † |
|---|---|---|---|---|---|
| Blank | 0 | 0 | 0 | 0 | 0 |
| Standard 1 | 5 | 0.1 | 0.1 | 1 | 0.1 |
| Standard 2 | 10 | 0.5 | 0.5 | 5 | 1 |
| Standard 3 | 20 | 1 | 1 | 10 | 5 |
| Standard 4 | 50 | 2 | 2 | 20 | 10 |

† Added for the DTPA-sorbitol method.

2. Dispense 20 mL of the DTPA or DTPA-sorbitol extracting solution into each extraction flask.
3. Shake the samples for 2 h using a reciprocating shaker at 180 oscillations per minute or a rotary shaker at 180 revolutions per minute.
4. Filter the suspension using Whatman No. 2 filter paper and collect the filtrate in vials for anaysis. Refilter if filtrate is cloudy.
5. A measurement of 20 g of soil and 40 mL of extracting solution may be used if not enough filtrate is acquired from soils with high clay content.

Analysis

1. Calibrate the ICP-AE spectrophotometer using multiple element standards following manufacturer's recommendations for the operation and calibration of the instrument.
2. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

## Calculations

1. The following formulas convert micronutrient concentrations from mg $L^{-1}$ in the extract to concentration units in soil on weight (mg $kg^{-1}$) or area basis (lbs $acre^{-1}$).

   a. Weight basis

   Weight basis, mg $kg^{-1}$ =

   mg $L^{-1}$ × (0.020 L extractant ÷ 10 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 2

   b. Area basis

   Nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula with a 6 inch sampling depth and assuming soil weight in an acre of soil at 6 inch depth is 2 million pounds.

   Area basis, lbs $acre^{-1}$ =

   mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.020 L extractant ÷ 10 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 4

## Analytical Performance

Range and Sensitivity

1. Instrument sensitivities allow reporting Fe, Zn, Mn, Cu, and B to the nearest 0.01 mg $kg^{-1}$.
2. Since micronutrient concentrations are low, detection limits may be close to the concentrations in the extracts. Detection limits should be quantified to ensure reported results are accurate.
3. Analytical results are only valid to the concentration of the highest calibration standard. If concentrations exceed these values, the sample should be diluted and reanalyzed. Alternatively, another calibration standard can be prepared at a concentration higher than the highest standard if the standard curve remains linear or is not extremely curvilinear.

Precision and Accuracy

1. Accuracy and precision of the method is dependent on several factors including fineness of soil pulverization, sample weight taken for extraction, and ICP configuration. Table 2 provides typical long-term statistics of intralaboratory precision from 1000 analyses of a quality control sample for DTPA extraction with modest to high levels of Fe, Zn, Mn and Cu and 30 analyses of B from a check sample with DTPA-sorbitol extraction. All analyses were performed using an axial ICP with cross-flow nebulizer.

*Table 2. Intralaboratory precision for micronutrients in DTPA (Fe, Zn, Mn, and Cu) or DTPA-sorbitol (B) extraction. Means and standard deviations are reported for 1000 analyses of a check sample for Fe, Zn, Mn, and Cu and 30 analyses of a check sample for B.*

| | Fe | Zn | Mn | Cu | B |
|---|---|---|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | | | | |
| Mean | 43.07 | 1.60 | 51.90 | 0.68 | 0.50 |
| Std. dev. | 4.753 | 0.278 | 7.893 | 0.084 | 0.04 |

Interferences

1. The use of modern charged coupled device detectors (CCD) in ICP-AE spectrophotometers has largely eliminated interference issues with DTPA analysis. While Zn interference on Cu lines occurred in older photomultiplier tubes of ICP-AE spectrophotometers, modern CCD instruments afford multiple interference free lines. The limiting factor in DTPA analysis on an ICP is the presence of significant concentrations of organic carbon. During introduction into the plasma, significant $CO_2$ is released and could result in line interferences if the laboratory is using wavelength lines less than 400 nm. The organic carbon should not pose an issue for metal analysis at wavelengths in the visible spectrum greater than 400 nm.
2. Low level detection of Cu can sometimes be challenging due to the matrix and its tendency to solubilize soil organic carbon compounds. While well-tuned radial ICP-AE spectrophotometers are capable of achieving reporting limits below 0.05 mg $L^{-1}$ Cu, many laboratories prefer to utilize axial ICP-AE spectrophotometers in order to ensure low detection levels of Cu. The soluble carbon species have a strong tendency to coat the aspiration tube of axial torches and many laboratories find they must switch out or clean

torches every 150 to 350 samples of DTPA extract filtrates or prior to analyzing other matrices.

## Interpretation

1. Regional or sub-regional correlation and calibration are required to utilize any soil test method. Table 3 provides an initial starting point for laboratories to consider for interpreting laboratory results.

*Table 3. Recommended soil fertility indices from DTPA or DTPA-sorbitol extraction of soil for Fe, Zn, Mn, Cu, and B.*

| Rating | Fe | Zn | Zn † (corn & sorghum) | Mn | Cu | B |
|---|---|---|---|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | | | | | |
| Extremely Low | | <0.1 | <0.1 | | | |
| Very Low | <2.19 | 0.1-0.18 | 0.1-0.31 | >0.33 | <0.05 | |
| Low | 2.19-3.19 | 0.18-0.22 | 0.31-0.60 | 0.33-0.66 | 0.05-0.1 | <0.25 |
| Medium | 3.19-4.19 | 0.22-0.27 | 0.60-0.80 | 0.66-1.00 | 0.1-0.16 | 0.25-0.5 |
| High | >4.19 | >.27 | >.80 | >1.00 | >0.16 | >0.5 |

† Corn and sorghum have higher soil test zinc requirements than most other agronomic crops.

## Storage

1. The DTPA solution and standards are relatively stable provided the laboratory takes steps to prevent evaporation and limits exposure of the solution to sunlight. The standards and extraction solutions can be stored up through 5 weeks. The presence of sorbitol in the DTPA-sorbitol extract can limit shelf life of the standards and extractant due to potential microbial growth. Microbial inhibitors, such as chloroform and toluene, can be added at 1 mL per L of extractant to limit growth.
2. Good laboratory practice strongly encourages immediate analysis of soil filtrates following extraction. Analysis completed within 48 h of extractions has been documented to be appropriate with limited precipitation of analytes. Refrigeration of samples to reduce microbial growth is recommended if filtrates are not analyzed the day of extraction.
3. Small quantities of white precipitates will likely remain in the bottom of the carboy when creating the DTPA or DTPA-sorbitol extract. This material is insoluble contaminants from the DTPA reagent and will not pose an analytical issue provided the precipitates are not brought into suspension during the dispensing operation. The extractant can be filtered if precipitates become a problem during dispensing. The carboy should be rinsed and acid washed before creating a new batch of extractant.

## Safety and Disposal

1. Once mixed, the DTPA extractant is relatively safe to handle, although good laboratory safety practices should always be employed. The extract can be flushed down common sewer systems with adequate freshwater.

## References

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*Chapter 4.6*

# Acetate and Mehlich-3 Extractable Sulfate-Sulfur

*D.K. Joines and D.H. Hardy*

## Application and Principle

Sulfur (S) is an essential macronutrient for plants. Important compounds in plants containing S include vitamin B, biotin, thiamine, and several amino acids. In general, the C:N:S ratio in plants is about 100:10:1. Sources of sulfur to the agroecosystem include soil minerals, atmospheric deposition, organic matter, and agrochemicals. Most S in surface soil is present as organic compounds. Organic-S can be mineralized by microorganisms to form sulfate-S ($SO_4^{2-}$) which is the form utilized by plants. Sandy soils in humid climates are usually low in S due to low soil organic matter content.

In the Southeastern US, two methods are utilized for extracting sulfate from soils. Tennessee and South Carolina use an extractant containing 0.5 *M* ammonium acetate and 0.25 *M* acetic acid (Bardsley and Lancaster, 1960). North Carolina uses Mehlich-3 (Mehlich, 1984). Each method incorporates inductively coupled plasma-atomic emission spectrometry (ICP-AES) for analysis of S.

## Equipment and Apparatus

Ammonium acetate/acetic acid method

1. Calibrated 5 g scoop or analytical balance with 0.01 g resolution
2. 50 ml extraction bottles or flasks with stoppers
3. Calibrated liquid dispenser to deliver 20 mL of extractant
4. Whatman 42 filter paper
5. Filter funnels and vials for receiving filtrates
6. Reciprocating mechanical shaker capable of 180 oscillations per minute
7. ICP-AE spectrometer

Mehlich-3 method

1. Soil scoop with 2.5 $cm^3$ if measuring soil by volume
2. Analytical balance with 0.01 g resolution if measuring soil be weight
3. Reciprocating mechanical shaker capable of 180 oscillations per minute
4. Volume dispenser to deliver 20 mL of Mehlich-3 extractant
5. Extraction bottles or flasks (50 to 100 mL) with stoppers
6. Filter funnels and vials for receiving filtrates
7. Whatman No. 1 filter paper or equivalent
8. ICP-AE spectrometer

## Reagents

Ammonium acetate/acetic acid method

1. *Ammonium acetate and acetic acid extractant*: Following are directions for making 2 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extractant. The solution contains 0.5 *M* ammonium acetate and 0.25 *M* acetic acid.
   a. In a 2 L volumetric flask, add about 1500 mL of deionized water.
   b. Add 77.0 g of ammonium acetate ($CH_3COONH_4$) and thoroughly stir to dissolve.
   c. Add 28.4 mL, or 29.8 g, of glacial acetic acid ($CH_3COOH$) and thoroughly stir to dissolve.
   d. Bring solution to 2 L volume with deionized water and thoroughly stir.
2. *Calibrations Standards*
   a. From a commercially purchased standard solution containing 1,000 mg $L^{-1}$ S, prepare calibration standards of 2 and 8 mg $L^{-1}$ S in the ammonium acetate and acetic acid extractant. Also prepare a blank calibration standard with 0 mg $L^{-1}$ S that just contains the extractant.

Mehlich-3 method

1. *Mehlich-3 extractant*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 0.2 *M* acetic acid, 0.25 *M* ammonium nitrate, 0.015 *M* ammonium fluoride, 0.013 *M* nitric acid, and 0.001 *M* ethylenediaminetetraacetic acid.

   Stock solution with 3.75 *M* $NH_4F$ and 0.25 *M* EDTA

   b. In a 2 L volumetric flask, add about 1200 mL $H_2O$
   c. Add 277.8 g ammonium fluoride ($NH_4F$) and thoroughly stir to dissolve.
   d. Add 146.1 g of ethylenediaminetetraacetic acid (EDTA, $((HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2)$ and thoroughly stir to dissolve.
   e. Bring solution to 2 L volume with deionized water and thoroughly stir.
   f. Store stock solution in plastic bottle.

   Mehlich-3

   g. Fill a 20-L calibrated plastic carboy with approximately 12 L of deionized water.
   h. Add 400 g of ammonium nitrate ($NH_4NO_3$) and thoroughly stir to dissolve.
   i. Add 80 mL of stock solution with 3.75 *M* $NH_4F$ and 0.25 *M* EDTA. Thoroughly stir to dissolve.
   j. Add 230 mL concentrated glacial acetic acid ($CH_3COOH$) and 16.4 mL concentrated $HNO_3$. Thoroughly stir.
   k. Bring solution to 20-L with deionized water and thoroughly stir.
   l. The pH of the extracting solution is approximately 2.5.
2. *Calibrations Standards*
   a. From a commercially purchased standard solution containing 1,000 mg $L^{-1}$ S, prepare calibration standards of 1.5, 15, 30 and 60 mg $L^{-1}$ S in Mehlich-3 extractant. Also prepare a blank calibration standard with 0 mg $L^{-1}$ S that just contains the Mehlich-3 extractant.

## Procedure

Ammonium acetate/acetic acid method

1. Measure 5 g processed soil (dried, < 2 mm) into a 50-mL extraction flask.
2. Add 20 mL of ammonium acetate and acetic acid extractant to the 50-mL bottle extraction flask.
3. Add stoppers to flasks and shake soil and Mehlich-3 for 5 min on a reciprocating mechanical shaker with a minimum of 180 oscillations per minute.
4. Filter the suspension using Whatman No. 1 filter paper and collect the filtrate for analysis.
5. The original method developed by Bardsley and Lancaster (1960) has 10 g soil and 25 mL of extractant shaken for 30 minutes prior to filtration. The soil/solution ratio and time of shaking was modified from the original method to better suit the variety of soils in Tennessee.

Mehlich-3 method

1. Measure 2.5 $cm^3$ processed soil (dried, < 2 mm) into a 50-mL extraction vessel.
2. Add 25 mL of Mehlich-3 extractant to the 50-mL extraction vessel.
3. Twenty-five mL of Mehlich-3 extractant and 2.5 $cm^3$ of soil, with a volume ratio of 10:1, were used in the originally developed Mehlich-3 method (Mehlich, 1984). As the Mehlich-3 extract became adopted by other laboratories, a ratio of 10:1 was used with soil measured on a weight basis. For example, 20 mL of Mehlich-3 and 2 g of soil are commonly used quantities. The weight of soil is either measured with a balance or approximated with a scoop of 1.7 $cm^3$ which assumes density of processed soil is 1.18 g $cm^{-3}$ (Peck, 1998). Whether using a 10:1 ratio based on soil volume or weight, fertilizer recommendations from the test results should be based on calibrations that used soil test results with the same ratio.
4. Add stoppers to flasks and shake soil and Mehlich-3 for 5 min on a reciprocating mechanical shaker with a minimum of 180 oscillations per minute.
5. Filter the suspension using Whatman No. 1 filter paper and collect the filtrate for analysis.

Analysis

1. Calibrate the ICP-AE spectrophotometer using multiple element standards following manufacturer's recommendations for the operation and calibration of the instrument.
2. An optimal wavelength for S sensitivity 181.975 nm. Below 190 nm, oxygen lines can interfere with S analysis. By utilizing a high purge rate of argon or nitrogen to remove oxygen, S analysis and detection limit are improved at the low wavelength (Boss and Fredeen, 2004).
3. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

## Calculations

Ammonium acetate/acetic acid method

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the ammonium acetate and acetic acid extract to concentration units in soil on a weight (mg $kg^{-1}$) or area basis (lbs $acre^{-1}$).

    a. Weight basis

    Weight basis, mg $kg^{-1}$ =

    mg $L^{-1}$ × (0.020 L extractant ÷ 5 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 4

    b. Area basis

    Nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula for 6 inch sampling depth with the assumption that soil weight in an acre of soil at 6 inch depth is 2 million pounds.

    Area basis, lbs $acre^{-1}$ =

    mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.020 L extractant ÷ 5 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 8

Mehlich-3 method

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the Mehlich-3 extract to various concentration units in soil on a volume (mg $dm^{-3}$), weight (mg $kg^{-1}$), or area basis (lbs $acre^{-1}$).

    a. Volume basis

    Mehlich (1984) developed the Mehlich-3 method using a scooped volume of processed soil to report nutrient concentration on a volume basis as shown below.

    Volume basis, mg $dm^{-3}$ =

    mg $L^{-1}$ × (0.025 L Mehlich-3 ÷ 2.5 $cm^3$ soil) × (1000 $cm^3$ ÷ 1 $dm^3$) = mg $L^{-1}$ × 10

    If the density of processed soil is assumed to be 1 g $cm^{-3}$, mg $dm^{-3}$ is equivalent to mg $kg^{-1}$.

    b. Weight basis

    For the alternative method of measuring soil by weight, such as using 20 mL Mehlich-3 and 2 g soil, the concentration in soil as mg $kg^{-1}$ is determined with the following formula.

    Weight basis, mg $kg^{-1}$ =

    mg $L^{-1}$ × (0.020 L Mehlich-3 ÷ 2 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 10

    The weight of soil is either measured or approximated with a 1.7 $cm^3$ scoop that assumes density of processed soil is 1.18 g $cm^{-3}$. This is an average density for silt loam soils.

c. Area basis

When measuring soil by weight, nutrient concentrations in units of lbs $acre^{-1}$ can be calculated for a 6 inch sampling depth assuming soil weight in an acre of soil at 6 inch depth is 2 million pounds.

Area basis, lbs $acre^{-1}$ =

mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.020 L Mehlich-3 ÷ 2 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 20

If measuring soil by volume with a Mehlich-3 to soil ratio of 10:1, the same factor of 20 can be used if the processed soil density is assumed to be 1 g $cm^{-3}$.

## Analytical Performance

Range and Sensitivity

1. The approximate detection limit for S at 181.975 nm with high purge oxygen removal and radial plasma torch orientation in the ammonium acetate/acetic acid extract is 0.1 mg $L^{-1}$.
2. The approximate detection limit for S at 182.040 nm with radial torch orientation in the Mehlich-3 extract is 0.2 mg $L^{-1}$.

Precision and Accuracy

1. Intralaboratory precision for sulfur analyses in the ammonium acetate/acetic acid and Mehlich-3 methods are shown in Table 1.

*Table 1. Intralaboratory precision of $SO_4$-S analysis via ammonium acetate/acetic acid and Mehlich-3 methods on control soils from the University of Tennessee and North Carolina Department of Agriculture and Consumer Services, respectively.*

| Sample | Number of measurements | Mean | Standard deviation |
|---|---|---|---|
| | Ammonium acetate/acetic acid method | | |
| | | ---------- mg $kg^{-1}$ ---------- | |
| SRS - 1101 | 10 | 54.3 | 1.5 |
| SRS - 1114 | 10 | 49.0 | 1.1 |
| | Mehlich-3 method | | |
| | | ---------- mg $dm^{-3}$ ---------- | |
| Clay loam | 96 | 2.3 | 0.5 |
| Loamy sand | 88 | 1.7 | 0.2 |

Interferences

1. Oxygen interferes with optimal detection of sulfur molecules at wavelengths less than 190 nm. Since S has greatest sensitivity at 181.975 nm, a high purge system with argon or nitrogen is required to remove excess oxygen from the chamber.

## Interpretation

1. Interpretation of S analysis of surface soil is not well established. The extraction procedures outlined here only extract soluble sulfate-S from soil. A significant portion of S that becomes available to plants during a growing season comes from mineralization of soil organic matter. Therefore, results from these methods are more useful for predicting S availability in sandy soils with low organic matter. For silt loam and clay soils with moderate to high organic matter, test results may do a poor job of predicting plant-available S during the growing season.
2. Tennessee and South Carolina use the ammonium acetate/acetic acid method. North Carolina uses the Mehlich-3 method. In Tennessee, recommendations for surface soils are made on a case by case basis due to varied responses by soil type and cropping systems across the state. In South Carolina, sulfur is considered deficient when concentrations are less than 10 mg $kg^{-1}$ in surface soil and less than 20 mg $kg^{-1}$ in subsoil. In North Carolina, S is recommended on agronomic crops, commercial vegetable crops, pastures and forage crops, and athletic turf when sulfur in surface soil is less than 12 mg $dm^{-3}$.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting results.
2. The ammonium acetate/acetic acid extractant should be used 1 month after preparation.
3. The Mehlich-3 extraction solution is stable and can be stored for several weeks due to its acidic nature. A specific shelf life is not known.

## Safety and disposal

1. The chemicals used in this procedure pose no safety risk with safe handling procedures. Chemicals should be stored and disposed of according to routine laboratory procedures.
2. Some labs may require the acidity of the Mehlich-3 extracts be neutralized before discarding into the sink.
3. It is advisable to remove the bulk of soil particles from the waste stream before discharging.

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*Chapter 4.7*

# Hot-Water Extractable Boron

*L. Sonon, D.E. Kissel, and F.J. Sikora*

## Application and Principle

Boron (B) is a naturally occurring element that is found in nature in the form of borates in the oceans, sedimentary rocks, coal, shale, and some soils. Boron is an essential micronutrient required for the normal growth of plants. The amount of total B in soils may range from 7 to 80 mg $kg^{-1}$ but the total B content in soils is not necessarily correlated with its availability to plants (Keren, 1996). In most soils, the amount of soil B available for plant uptake is less than 5% of total B in soil (Gupta, 1967).

Extraction of soil B with hot-water was originally developed by Berger and Troug (1939) which involves refluxing soil with hot-water for 5 min using a soil/water ratio of 1:2. The procedure was modified by Gupta (1967) who found that increasing the refluxing time from 5 to 10 min resulted in a significant increase in the amount of B extracted.

The extracted B can be analyzed colorimetrically using reagents such as carmine (Hatcher and Wilcox, 1950) or Azomethine-H (Wolf, 1971). The most commonly used method in recent years is analysis via inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Keren, 1996). The latter is the method of choice among laboratories in the Southeastern region of the US because it has a wider dynamic concentration range and is a quicker method for analysis. Gestring and Soltanpour (1981) found a good correlation between B determination by ICP-AES and the Azomethine-H for soil extracts.

Over the years, some laboratories have made modifications to the procedure developed by Berger and Troug (1939). The University of Kentucky and Louisiana State University use the original extraction method of Berger and Troug (1939). The University of Georgia modified the extraction using a 1:5 soil/water ratio and shaking for 30 min in a hot-water bath at 80$^{o}$C. The methods below describe both extraction procedures with analysis of B in the extract via ICP-AES.

## Equipment and Apparatus

<u>Boiling hot-water method</u>

1. Calibrated 10 g scoop or analytical balance with 0.01 g resolution for measuring soil
2. 500 mL Erlenmeyer flasks with tapered outer joint for fitting a reflux condenser
3. Reflux condenser columns that fit into outer joint of 500 mL Erlenmeyer flasks
4. Dispenser to deliver 20 mL of deionized water or 0.01 *M* $CaCl_2$
5. Hotplates to heat a single 500 mL Erlenmeyer flask
6. Aluminum plates to shield heat from Erlenmeyer flasks to cool contents after boiling
7. Plastic funnels and plastic containers for receiving filtrates
8. One 1-L volumetric flask and 3 100-mL volumetric flasks, plastic preferred
9. Whatman No. 2 filter paper
10. ICP-AE spectrometer

80°C hot-water method

1. Calibrated 5-g scoop or balance with 0.01 g resolution
2. Dispenser to deliver 25 mL deionized water or 0.01 *M* $CaCl_2$
3. Plastic funnels and plastic containers for receiving filtrates
4. 125-mL plastic Erlenmeyer flasks for extraction and receiving filtrates
5. Whatman No. 1 filter paper
6. One 1-L volumetric flask and 3 100-mL volumetric flasks, plastic preferred
7. Reciprocating hot-water shaking bath
8. ICP-AE spectrometer

## Reagents

Boiling hot-water method or 80°C hot-water method

1. *Calcium Chloride ($CaCl_2$), 0.01 M* : This reagent can be used in place of water as an extractant to reduce turbidity in filtrates. Add 1.47 g of $CaCl_2 \cdot 2H_2O$ to a 1-L volumetric flask. Make to volume with deionized water and stir well. Transfer solution to a plastic bottle for storage. It is advisable to use a plastic volumetric flask to avoid B contamination from glassware. If using a glass flask, immediately transfer contents to a plastic bottle for storage.
2. *B Stock Solution (1000 mg $L^{-1}$)*: Use National Institute of Standards and Technology (NIST) traceable single element plasma grade standard.
3. *B Stock solution (10 mg $L^{-1}$)*: Pipette 10 mL of the 1000 mg $L^{-1}$ B stock solution into a 1-L volumetric flask. Make to volume with deionized water and mix well. If the volumetric flask is plastic, the standard can be stored in this container or transferred to a plastic bottle. If the volumetric flask is glass, transfer the solution to a plastic bottle for storage to avoid B contamination from glassware.

Boiling hot-water method

4. *Calibration Standards for ICP-AES*: Pipette 5 and 15 mL of the 10 mg $L^{-1}$ B stock solution to 100 mL volumetric flasks and make to volume with deionized water to create standards of 0.5 and 1.5 mg $L^{-1}$ B, respectively. Use deionized water as a blank. If the volumetric flasks are plastic, the standards can be stored in these containers or transferred to plastic bottles. If the volumetric flasks are glass, transfer the solutions to plastic bottles for storage to avoid B contamination from glassware. If using 0.01 *M* $CaCl_2$ as an extractant rather than water, use 0.01 *M* $CaCl_2$ to make to volume and as a blank.

80°C hot-water method

5. *Calibration Standards for ICP-AES*: Pipette 1 and 3 mL of the 10 mg $L^{-1}$ B stock solution to 100 mL plastic volumetric flasks and make to volume with deionized water to create standards of 0.1 and 0.3 mg $L^{-1}$ B, respectively. Use deionized water as a blank. If the volumetric flasks are plastic, the standards can be stored in these containers or transferred to plastic bottles. If the volumetric flasks are glass, transfer the solutions to plastic bottles for storage to avoid B contamination from glassware. If using 0.01 *M* $CaCl_2$ as an extractant rather than water, use 0.01 *M* $CaCl_2$ to make to volume and as a blank.

## Procedure

Boiling hot-water method

1. Scoop or weigh 10 g of processed soil (dried, <2 mm) into 500-mL Erlenmeyer flasks. If the soil is measured with a scoop, assume a density of 1.18 g $cm^{-3}$ for fine-textured soils and use an 8.5 $cm^3$ scoop. For coarse-textured soils, assume a density of 1.25 g $cm^{-3}$ and use an 8 $cm^3$ scoop.
2. Add 20 mL deionized water with a dispenser. Deionized water may be substituted with 0.01 *M* $CaCl_2$ to reduce turbidity in the filtrate with no significant change in extractable B (Jeffery and McCallum, 1988; Watson, 1988).
3. Place Erlenmeyer flasks on hot plates. Attach reflux condenser columns to top of flasks.
4. Turn the heat on. Carefully watch solutions as they heat up. When boiling begins, turn heat down to low.
5. Allow solution to boil for 10 min then turn heat off. Place aluminum plates in between flasks and hot plate to facilitate solution cooling.
6. After solutions have cooled, filter the soil slurry using Whatman No. 2 filter paper and plastic funnels. Collect filtrate in plastic containers. The supernatant may be turbid due to colloidal materials that pass through the filter, check the filtrate for clarity and refilter if necessary.
7. Calibrate the ICP-AE spectrophotometer with a 0, 0.5, and 1.5 mg $L^{-1}$ B standards.
8. Use the 0.5 mg $L^{-1}$ standard as a curve verification check. Analyze this standard immediately after calibration and after the last soil sample.
9. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

80°C hot-water method

1. Scoop or weigh 5 g of processed soil (dried, <2 mm) into 125-mL plastic flasks. If the soil is measured with a scoop assume a density of 1.18 g $cm^{-3}$ for fine-textured soils and use a 4.25 $cm^3$ scoop. For coarse-textured soils, assume a density of 1.25 g $cm^{-3}$ and use a 4 $cm^3$ scoop.
2. Add 25 mL deionized water with a dispenser. Deionized water may be substituted with 0.01 *M* $CaCl_2$ to reduce turbidity in the filtrate with no significant change in extractable B (Jeffery and McCallum, 1988 and Watson, 1988).
3. Load the flasks on a reciprocating hot-water shaking bath and shake the slurries for 30 min at 80°C.
4. Filter the soil slurry using Whatman No. 1 filter paper and plastic funnels. Collect filtrate in 125-mL plastic Erlenmeyer flasks. The supernatant may be turbid due to colloidal materials that pass through the filter, check the filtrate for clarity and refilter if necessary.
5. Calibrate the ICP-AE spectrophotometer with a 0, 0.10, and 0.30 mg $L^{-1}$ B standards.
6. Use the 0.10 mg $L^{-1}$ standard as a curve verification check. Analyze this standard immediately after calibration and after the last soil sample.
7. Analyze the unknown solution extracts. The solution extract should be diluted if the sample concentration exceeds the concentration of the highest standard.

## Calculations

Boiling hot-water method

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the extract to various concentration units in soil on a weight (mg $kg^{-1}$) or area basis (lbs $acre^{-1}$).

    a. Weight basis
    Weight basis, mg $kg^{-1}$ =
    mg $L^{-1}$ × (0.020 L extractant ÷ 10 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 2

    b. Area basis
    When measuring soil by weight, nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula with assumptions that soil weight in an acre of soil at 6 inch depth is 2 million pounds.

    Area basis, lbs $acre^{-1}$ =
    mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.020 L extractant ÷ 10 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 4

80°C hot-water method

1. The following formulas convert nutrient concentrations from mg $L^{-1}$ in the extract to various concentration units in soil on a weight (mg $kg^{-1}$) or area basis (lbs $acre^{-1}$).

    c. Weight basis
    Weight basis, mg $kg^{-1}$ =
    mg $L^{-1}$ × (0.025 L extractant ÷ 5 g soil) × (1000 g soil ÷ kg soil) = mg $L^{-1}$ × 5

    d. Area basis
    When measuring soil by weight, nutrient concentrations in units of lbs $acre^{-1}$ can be calculated according to the following formula with assumptions that soil weight in an acre of soil at 6 inch depth is 2 million pounds.

    Area basis, lbs $acre^{-1}$ =
    mg $L^{-1}$ × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.025 L extractant ÷ 5 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ × 10

## Analytical Performance

Range and Sensitivity

1. The detection limit for B with ICP-AES at 249.6 nm is typically 0.01 mg $L^{-1}$ B. Achieving a reliably low detection limit is important since the agronomic critical value for soil test B is low. For the boiling hot-water method, 0.01 mg $L^{-1}$ B corresponds to 0.02 mg $kg^{-1}$ in soil. For the 80°C hot-water method, 0.01 mg $L^{-1}$ B corresponds to 0.05 mg $kg^{-1}$ in soil.

Precision and Accuracy

1. Soil samples from the North American Proficiency Testing (NAPT) Program and one quality control (QC) sample were analyzed with the boiling and 80°C hot-water methods (Table 1). Analyses were performed with eight replications at the University of Kentucky (KY) and the University of Georgia (GA). The intralaboratory precision (std. dev.) was better than the interlaboratory precision (NAPT MAD) from the NAPT program as expected.

*Table 1. Intralaboratory and interlaboratory precision of soil B analysis via boiling and 80°C hot-water extraction methods on NAPT and QC soils. Boiling hot-water method and 80°C hot-water method were performed on 8 replications at the University of Kentucky and the University of Georgia, respectively.*

| | Boiling hot-water method | | | |
|---|---|---|---|---|
| Soil | KY lab median | KY lab std. dev. | NAPT median | NAPT MAD |
| | ---------- mg kg$^{-1}$ ---------- | | | |
| NAPT 2003-101 | 0.450 | 0.049 | 0.370 | 0.080 |
| NAPT 2004-108 | 0.350 | 0.071 | 0.360 | 0.130 |
| NAPT 2005-101 | 0.870 | 0.101 | 0.730 | 0.180 |
| NAPT 2005-104 | 0.275 | 0.073 | 0.240 | 0.080 |
| | 80°C hot-water method | | | |
| | GA lab median | GA lab std.dev. | NAPT median | NAPT MAD |
| | ---------- mg kg$^{-1}$ ---------- | | | |
| NAPT 2009-101 | 0.535 | 0.026 | 0.500 | 0.128 |
| NAPT 2009-104 | 0.226 | 0.010 | 0.200 | 0.040 |
| NAPT 2009-116 | 0.685 | 0.046 | 0.715 | 0.225 |
| UGA QC Soil | 0.093 | 0.025 | | |

2. For routine soil B analysis, laboratories may establish limits of acceptability. For example, duplicate values reading within 20% of the average of the two values may be acceptable.
3. The low levels of B extracted from soil poses a challenge on obtaining accurate and precise results as the measured value approaches the instrument's detection limit. The situation is exacerbated for agronomic interpretation since the critical value is close to the detection limit where precision of analysis worsens. Therefore, B analysis of plant tissue is equally or even more important than soil analysis.

Interferences

1. Care must be taken to obtain clear filtrates without any soil colloids to avoid clogging the nebulizer in the ICP-AE spectrometer.
2. If glassware is used, it should be washed with a 1:1 mixture of boiling HCl and deionized water before use to leach out trace B in the glass. It is also advisable to run replicate analysis

of all samples when using glassware to isolate random contamination errors due to insufficient leaching and removal of B from the glass.

3. Background B that can come from labware and filter paper should be periodically checked by running a method blank in a set of samples which include all the procedural steps except the addition of soil. If the B concentration in the method blank is greater than the detection limit, the concentration should be subtracted from sample concentrations or the source of the contamination should be determined and eliminated.

## Interpretation

1. The Universities of Kentucky and Georgia use the following soil fertility indices for hot-water extractable B. The Kentucky indices are used for alfalfa and the Georgia indices are used for alfalfa, cotton, peanuts, vegetable crops, and clover. The difference in the indices may be due to different methods of extraction or the differences in soils existing in each state with fine-textured soils in Kentucky and coarse-textured soils in Georgia. Ouellette and Lachance (1954) observed fine-textured soils to have higher agronomic critical values compared to coarse-textured soils for alfalfa.

*Table 2. Recommended soil fertility indices for hot-water extraction of soil for B. Indices for the boiling hot-water method are from the University of Kentucky (KY). Indices for the 80$^{o}$C hot-water method are from the University of Georgia (GA).*

| Index | KY boiling hot-water | GA 80$^{o}$C hot-water |
|---|---|---|
| | ---------- mg kg$^{-1}$ ---------- | |
| Low | <0.3 | <0.15 |
| Medium | 0.3-1 | 0.16-0.5 |
| High | >1 | >0.5 |

2. In Kentucky, 1.5 to 2 lbs acre$^{-1}$ B is recommended for alfalfa every other year. In Georgia, 0.25 to 2 lbs acre$^{-1}$ boron is recommended for alfalfa, cotton, soybeans, peanuts, vegetable crops, and clover. It is not advisable to exceed the recommended rates since B toxicity can occur from excessive application. When soil test B level exceeds 1 mg kg$^{-1}$ in Kentucky or 0.5 mg kg$^{-1}$ in Georgia, B application is not recommended. Crops differ in their sensitivity to B toxicity. Sensitive crops are soybeans, peaches, and strawberries. Some of the tolerant crops are alfalfa, clovers, cole crops, and root crops. Corn, cotton, tobacco, tomatoes and small grains are intermediate.

## Effects of Storage

1. Glassware contains borosilicate that can release B into solution. For accurate and reproducible analyses at low B concentrations, it is important to use non-borosilicate containers to collect leachate and store ICP-AES standards.

## Safety Disposal

1. The chemicals used in this procedure pose no safety risk and therefore can be stored and disposed of according to routine laboratory procedures.

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*Chapter 4.8*

# Nitrate-Nitrogen

*R. Miller and L. Sonon*

## Application and Principle

Nitrogen (N) is a plant nutrient present in the highest concentration in plants and can often limit plant growth and yield. Nitrogen mostly exists in soil as part of soil organic matter. The N in soil organic matter is not available to plants until it is mineralized and released as ammonium ($NH_4^+$) which can further be transformed to nitrate ($NO_3^-$) with nitrification. The $NO_3^-$ is readily leached from soils in climates with abundant rainfall. There is not a quick and efficient soil test that can reliably predict the amount of N in soil organic matter that would be mineralized and made available to plants. Nitrate will remain in soil with climates of limited precipitation since there is limited loss due to leaching and denitrification. The $NO_3^-$ can be readily extracted from soil and analyzed. Soil sampling and analysis of $NO_3^-$ can occur any time of year. For climates having significant rainfall, sampling soil for $NO_3^-$ any part of the year does not provide useful information because of the high probability for $NO_3^-$ loss. There is a soil test for corn where $NO_3^-$ is determined in soil at the time when the crop is 8 to 12 inches tall and can be sidedressed with N fertilizer. This is close to the time when the crop will have maximum uptake of nutrients and soil $NO_3^-$ concentration at this time is a good indicator of $NO_3^-$ availability to the crop.

Two methods are described here for determining plant-available $NO_3^-$ in soil. The first is a method primarily used in climates with limited rainfall where $NO_3^-$ is not readily leached and remains in the soil. This method involves extraction of nitrate-nitrogen ($NO_3$-N) from soils using 2 *M* KCl. Nitrate is determined by reduction to nitrite ($NO_2$-N) via a cadmium reactor, diazotized with sulfanilamide and coupled to N-(1-Napthyl)-ethylenediamine dihydrochloride to form an azochromophore (red-purple in color) which is measured spectrophotometrically at 520 nm. The method is readily adapted to manual or automated techniques. The procedure follows the method outlined by Mulvaney (1996) for determining $NO_3$-N with a modification that 25 mL of 2 *M* KCl and 5 g of soil are used instead of 100 mL and 10 g soil, respectively. Since the measured $NO_3$-N is available for plant uptake in climates with low annual precipitation, the test result can be credited toward crop nitrogen needs.

The second method determines plant-available $NO_3^-$ at the time of sidedressing corn and is referred to as the presidedress nitrate test (PSNT). This method was developed in Vermont (Madgoff et al., 1984) with research on its applicability reported in Iowa (Blackmere et al., 1989) and Northeastern US (Magdoff et al., 1990; Meisinger et al., 1992; Klausner et al., 1993; Sims, et al., 1995). Soil is sampled at a 12 inch depth. The depth of sampling is deeper than sampling for other nutrients due to the mobility of $NO_3$-N in soil. The $NO_3$-N is extracted from soil with 0.04 *M* $(NH_4)_2SO_4$. The filtrate can be analyzed for $NO_3$-N via the spectrophotometric method described in the previous method. Alternatively, $NO_3$-N can be analyzed with an ion-selective electrode (ISE). Analysis via an ISE is not as accurate as the spectrophotometric analysis but it is quicker and a more convenient method of analysis when handling small sample numbers. The soil $NO_3$-N concentration provides information on how much N should be sidedressed to corn.

## Equipment and Apparatus

Nitrate extracted with KCl

1. Analytical balance with resolution of 0.01 g for measuring soil
2. Volume dispenser to deliver 25 mL of 2 *M* KCl extractant solution
3. Reciprocating mechanical shaker capable of 180 oscillations per minute
4. Extraction flasks or bottles
5. Filtration funnels and vessels to receive filtrates
6. Whatman No. 42 or equivalent highly retentive filter paper
   a. Check a lot of filter paper for possible contamination of $NO_3$-N by passing 25 mL of 2 *M* KCl through the filter paper. If contamination is greater than 0.2 mg $L^{-1}$, rinse filter paper with 2.0 *M* KCl before use.
7. Spectrophotometer, automated segmented flow analyzer, or flow injection analyzer capable of determining absorbance at 520 nm

PSNT with nitrate analysis via ISE

1. Analytical balance with resolution of 0.01 g for measuring soil
2. Volume dispenser to deliver 25 mL of 0.04 *M* $(NH_4)_2SO_4$ extractant solution
3. Reciprocating mechanical shaker capable of 180 oscillations per minute
4. Extraction flasks or bottles
5. Filtration funnels and vessels to receive filtrates
6. Whatman No. 42 or equivalent highly retentive filter paper
   a. Check a lot of filter paper for possible contamination of $NO_3$-N by passing 25 mL of 0.04 *M* $(NH_4)_2SO_4$ through the filter paper. If contamination is greater than 0.2 mg $L^{-1}$, rinse filter paper with 0.04 *M* $(NH_4)_2SO_4$ before use.
7. Spectrophotometer, automated segmented flow analyzer, or flow injection analyzer capable of determining absorbance at 520 nm if determining $NO_3^-$ via colorimetry
8. Nitrate ISE, double junction reference electrode, and pH/mV meter if determining $NO_3^-$ via potentiometry. The double junction reference electrode is not needed if the nitrate ISE has an internal reference electrode.

## Reagents

Nitrate extracted with KCl

1. *Potassium chloride extraction solution (2 M KCl)*:
   a. Add 150 g of reagent grade KCl to a 1000 mL volumetric flask.
   b. Add approximately 800 mL of deionized water and stir to dissolve.
   c. After solid is dissolved, add deionized water to bring the volume to 1000 mL.
   d. Soils can also be extracted with 1 *M* KCl (Kachurina et al., 2000). To prepare 1 *M* KCl, follow the directions previously described using 75 g of reagent grade KCl.
2. *Calibration standards*: Prepare six calibration standards ranging from 0.1 to 20 mg $L^{-1}$ $NO_3$-N in the KCl extraction solution prepared from 1000 mg $L^{-1}$ $NO_3$-N standard solution.

PSNT with nitrate analysis via ISE

1. *Ammonium sulfate extraction solution (0.04 M $(NH_4)_2SO_4$)*:
   a. Add 5.28 g of reagent grade $(NH_4)_2SO_4$ to a 1000 mL volumetric flask.
   b. Add approximately 500 mL of deionized water and stir to dissolve.
   c. After solid is dissolved, add deionized water to bring the volume to 1000 mL.

2. *Ionic strength adjustor (ISA) (2 M $(NH_4)_2SO_4$)*:
   a. Add 26.4 g of reagent grade $(NH_4)_2SO_4$ to a 100 mL volumetric flask.
   b. Add approximately 80 mL of deionized water and stir to dissolve.
   c. After the solid is dissolved, add deionized water to bring the volume to 1000 mL.
3. *Preservative for calibration standards (1 M $H_3BO_3$)*:
   a. Dissolve 6.2 g of reagent grade $H_3BO_3$ in 100 mL of boiling water in a beaker placed on a hot plate.
   b. Let solution cool and transfer to a 100 mL volumetric flask. Make to volume with deionized water.
4. *Calibration standards*: Prepare 500 mL of three calibration standards of 0, 4, 10, and 40 mg $L^{-1}$ $NO_3$-N from a 1000 mg $L^{-1}$ $NO_3$-N standard solution. To each standard, add 10 mL of ISA and 5 mL of 1 *M* $H_3BO_3$ preservative. Dilute to volume with deionized water.

## Procedure

Nitrate extracted with KCl

1. Weigh 5 g of processed soil (dried, < 2 mm) into an extraction flask or bottle.
2. Add 25 mL of KCl extraction solution using a dispenser.
3. Place extraction vessels on reciprocating mechanical shaker at 180 oscillations per minute and shake for 1 h.
4. Filter the soil suspension and collect filtrate. Refilter if filtrate is cloudy.
5. Nitrate-N content of the filtrate is determined using a spectrophotometer, automated segmented flow analyzer (Technicon Method No. 329-74W/A) or flow injection analyzer instrument. Calibrate using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine nitrate concentrations in filtrates as mg $L^{-1}$ $NO_3$-N.

PSNT with nitrate analysis via ISE

1. Weigh 10 g of processed soil (dried, < 2 mm) into an extraction flask or bottle.
2. Add 25 mL of 0.04 *M* $(NH_4)_2SO_4$ extraction solution using a dispenser.
3. Place extraction vessels on reciprocating mechanical shaker at 180 oscillations per minute and shake for 15 min.
4. Filter the soil suspension and collect filtrate. Refilter if filtrate is cloudy.
5. Nitrate-N content of the filtrate is determined using a $NO_3^-$ ISE and pH/mV meter as described below.
   a. Dilute 2 mL of ISA to 100 mL in a volumetric flask. Add this solution to the outer chamber of the reference electrode.
   b. Place the ISE in the 0 mg $L^{-1}$ $NO_3$-N standard while stirring the solution with a magnetic stir bar at moderate speed. Keep the electrode in the blank standard for several minutes until reading has stabilized. Stir solutions with a magnetic stir bar at moderate speed when electrode is placed in all solutions for measurements.
   c. Calibrate the meter using the 4 and 40 mg $L^{-1}$ $NO_3$-N standards. Calibrate with the 4 mg $L^{-1}$ standard first.
   d. After calibration with 4 and 40 mg $L^{-1}$ $NO_3$-N, check the concentration of the 10 mg $L^{-1}$ standard.
   e. Place $NO_3$-N ISE into each of the soil filtrates with stirring to measure $NO_3$-N concentration. Record concentration in filtrates as mg $L^{-1}$ $NO_3$-N.

6. Nitrate-N content of the filtrate can be determined spectrophotmetrically as described for the method of extracting soil with KCl.

## Calculations

Nitrate extracted with KCl

1. The following formulas convert $NO_3$-N concentrations from mg $L^{-1}$ in the extract to various concentration units in soil on a weight (mg $kg^{-1}$) or area basis (lbs $acre^{-1}$).

    a. Weight basis
    Weight basis, mg $kg^{-1}$ $NO_3$-N =
    mg $L^{-1}$ $NO_3$-N × (0.025 L extractant ÷ 5 g soil) × (1000 g soil ÷ kg soil) =
    mg $L^{-1}$ $NO_3$-N × 5

    b. Area basis
    Nutrient concentrations in units of lbs $acre^{-1}$ can be calculated for 6 inch sampling depth assuming weight of soil in an acre at 6 inch depth is 2 million pounds.

    Area basis, lbs $acre^{-1}$ $NO_3$-N =
    mg $L^{-1}$ $NO_3$-N × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.025 L extractant ÷ 5 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($2\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ $NO_3$-N × 10

PSNT with nitrate analysis via ISE

1. The following formula converts $NO_3$-N concentrations from mg $L^{-1}$ in the extract to concentration on a weight basis of soil as mg $kg^{-1}$.

    a. Weight basis
    Weight basis, mg $kg^{-1}$ $NO_3$-N =
    mg $L^{-1}$ $NO_3$-N × (0.025 L extract ÷ 10 g soil) × (1000 g soil ÷ kg soil) =
    mg $L^{-1}$ $NO_3$-N × 2.5

    b. Area basis
    Nutrient concentrations in units of lbs $acre^{-1}$ can be calculated for 12 inch sampling depth assuming weight of soil in an acre at 12 inch depth is 4 million pounds.

    Area basis, lbs $acre^{-1}$ $NO_3$-N =
    mg $L^{-1}$ $NO_3$-N × (2.2 lbs nutrient ÷ $10^6$ mg nutrient) × (0.025 L extractant ÷ 10 g soil) × (1000 g soil ÷ 2.2 lbs soil) × ($4\times10^6$ lbs soil ÷ acre) = mg $L^{-1}$ $NO_3$-N × 10

## Analytical Performance (nitrate extracted with KCl)

Range and Sensitivity

1. Soil $NO_3$-N measurements can be made to the nearest 0.1 mg $kg^{-1}$.
2. Analytical results are only valid to the concentration of the highest calibration standard. If concentrations exceed these values, the sample should be diluted and reanalyzed.

Alternatively, another calibration standard can be prepared at a concentration higher than the highest standard if the standard curve remains linear or is not extremely curvilinear.

Precision and Accuracy

1. Typical measurements of intralaboratory precision for soil $NO_3$-N as measured by the cadmium reduction method (Mulvaney, 1996) in Flow Injection Analysis (FIA) are shown in the Table below.

*Table 1. Intralaboratory precision for soil $NO_3$-N with 2 M KCl extraction using FIA on three replications with soils from the 2010 Agricultural Laboratory Proficiency program (ALP, 2013).*

| Sample | Mean | Standard deviation |
|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | |
| SRS - 1003 | 4.6 | 0.3 |
| SRS - 1006 | 11.4 | 0.6 |
| SRS - 1008 | 24.6 | 0.9 |
| SRS - 1010 | 46.3 | 1.2 |

Interferences

1. Moist samples should be air-dried soon after sampling to avoid N transformations that can change the $NO_3$-N concentration in the soil.
2. Nitrite ($NO_2$-N) in soil will result in a positive interference. It is very unlikely that soil samples that have been thoroughly dried will contain $NO_2$-N. To determine $NO_2$-N concentration, repeat extraction and analyze the extract without the use of the cadmium reduction column.

## Analytical Performance (PSNT with nitrate analysis via ISE)

Range and Sensitivity

1. Soil $NO_3$-N measurements can be made to the nearest 0.1 mg $kg^{-1}$.
2. It is not that critical to dilute the sample if $NO_3$-N concentration in the extract exceeds the concentration of the highest calibration standard because ISEs have a good linear range and any concentration beyond 40 mg $L^{-1}$ $NO_3$-N is much greater than the agronomic critical value for recommendations.
3. The linear range of the $NO_3$-N ISE extends down to 1 mg $L^{-1}$ $NO_3$-N which corresponds to 2.5 mg $kg^{-1}$ $NO_3$-N in soil. Nitrate-N can be detected below this concentration but with much less sensitivity. The approximate detection limit is 0.1 mg $L^{-1}$ $NO_3$-N.

Precision and Accuracy

1. Table 2 shows information on intralaboratory precision for repeated measurements of $NO_3$-N with an ISE at the University of Kentucky on 3 soil samples from the North American Proficiency Testing (NAPT) program (NAPT, 2013). Interlaboratory precision from the NAPT program is also shown on the same samples. Intralaboratory precision should be less than interlaboratory precision which occurred on 2 of the 3 samples.

*Table 2. Intralaboratory and interlaboratory precision for soil $NO_3$-N with ISE on 3 soil samples from the NAPT program.*

| | Intralaboratory precision | | | Interlaboratory precision | | |
|---|---|---|---|---|---|---|
| NAPT Sample | No. | Avg. mg $kg^{-1}$ | St.dev. mg $kg^{-1}$ | No. of labs | Median mg $kg^{-1}$ | MAD mg $kg^{-1}$ |
| 2005 – 111 | 12 | 34.9 | 2.7 | 28 | 28.8 | 5.3 |
| 2005 – 115 | 16 | 17.7 | 2.6 | 28 | 19.5 | 4.9 |
| 2005 – 118 | 10 | 22.6 | 3.5 | 23 | 25.5 | 3.5 |

2. Interlaboratory precision for samples from the NAPT program (NAPT, 2013) is shown below with a comparison between ISE and spectrophotometric analysis of $NO_3$-N. The median absolute deviation (MAD) is greater with ISE which reveals the poorer precision for this method of analysis.

*Table 3. Interlaboratory precision for soil $NO_3$-N with ISE and spectrophotometric analyses on selected soil samples from the NAPT program.*

| | ISE | | | Spectrophotometry | | |
|---|---|---|---|---|---|---|
| NAPT Sample | No. of labs | Median mg $kg^{-1}$ | MAD mg $kg^{-1}$ | No. of labs | Median mg $kg^{-1}$ | MAD mg $kg^{-1}$ |
| 2002 – 108 | 22 | 11.5 | 2.9 | 61 | 9.4 | 0.6 |
| 2002 – 116 | 26 | 63.3 | 7.5 | 55 | 69.6 | 4.4 |
| 2003 – 102 | 25 | 23.4 | 3.0 | 54 | 27.8 | 1.4 |
| 2005 – 104 | 27 | 6.3 | 1.4 | 69 | 5.4 | 0.7 |

Interferences

1. Moist samples should be air-dried soon after sampling to avoid N transformations that can change the $NO_3$-N concentration in the soil.
2. Bicarbonate and chloride concentrations more than 44 and 76 times the concentration of nitrate, respectively, can have a positive interference resulting in 10% higher concentration of measured $NO_3$-N than is actually present.
3. The gel membrane in the sensing module of the ISE has a limited life expectancy. When response time becomes sluggish or the electrode drifts and does not maintain calibration, replace the old module with a new one.
4. The gel membrane in the sensing module absorbs $NO_3$-N which results in some carryover of nitrate when going from measuring a solution with high concentration to a solution with low concentration. When beginning analysis, soak the ISE in a blank standard for several minutes until reading has stabilized. Proceed and measure calibration standards from lowest concentration to highest concentration. Rinse electrode with deionized water in between standards and unknowns.

## Interpretation

Nitrate extracted with KCl

1. In climates with low annual precipitation, like parts of Texas and Oklahoma, nitrate will not leach and remain in soil. Thus, measured nitrate is considered available to plants during the growing season. For samples taken at a 6 inch depth, mg $L^{-1}$ $NO_3$-N is multiplied by 10 to obtain lbs $acre^{-1}$ $NO_3$-N. The measured lbs $acre^{-1}$ $NO_3$-N is deducted from the recommended amount of fertilizer N for the crop to be grown. For samples taken at different depths, a weight of soil other than 2 million pounds can be assumed for determining lbs $acre^{-1}$ $NO_3$-N

PSNT with nitrate analysis via ISE

1. A common critical value for corn response to sidedress N ranges from 20 to 25 mg $kg^{-1}$. The University of Kentucky and University of Tennessee use a value of 25 mg $kg^{-1}$. If $NO_3$-N is less than 25 mg $kg^{-1}$, there is a good probability that corn will respond to sidedress nitrogen application. The guideline for N application rates used at the Universities of Kentucky is shown in Table 4. The University of Tennessee uses similar recommendations in addition to having a higher critical value and higher N rates for field consistently yielding more than 175 bu $acre^{-1}$ (Savoy, 1999).

*Table 4. Recommended N application rates for sidedressing corn based on PSNT results used at the University of Kentucky.*

| Index | $NO_3$-N | Recommended N rate |
|---|---|---|
| | ---- mg $kg^{-1}$ ---- | ---- lbs $acre^{-1}$ ---- |
| Low | < 11 | 100 to 160 |
| Medium | 11 – 25 | 0 to 100 |
| High | > 25 | 0 |

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil nitrate provided they are stored in a moisture free environment.
2. The soil extracts should be analyzed soon after filtration. If soil extracts cannot be analyzed the same day as extraction, store the extracts at $4^oC$ or with 100 µL of toluene or thymol added to the extracts.

## Safety and disposal

1. The cadmium used for the reduction of nitrate to nitrite in the spectrophotometric method is a hazardous waste and needs to be disposed of accordingly.
2. Other chemicals used in this procedure pose no safety risk and therefore can be stored and disposed of according to routine laboratory procedures

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*Unit 5*

# Soil Organic Matter

*Chapter 5.1*

# Introduction to Soil Organic Matter

*F.J. Sikora and D.H. Hardy*

Soil organic matter consists of C containing compounds that were once part of living plant, animal, and microbial cells. The compounds exist in various stages of decomposition. Humus is a stable fraction of soil organic matter that is left over after extensive decomposition and is the material that gives soils a dark brown to black color.

The properties of soil organic matter offer significant benefits to crop production and soil quality. First, soil organic matter has high water holding capacity that can greatly enhance soil productivity. Secondly, the colloidal property of humus gives it extensive surface area that is responsible for high cation exchange capacity that is regulated by soil pH. The high cation exchange capacity helps prevent the leaching loss of positively charged plant nutrients such as Potassium (K) and Magnesium (Mg). The high negative charge on humus also holds various mineral solids together through metal ion bridging; this coagulation enhances the formation of stable soil aggregates that improve soil structure which improves water infiltration, water percolation, and root growth. Additionally, soil organic matter contains plant nutrients, primarily N, P, and S that enhance overall soil fertility and productivity.

The properties of soil organic matter are so significant that a relatively small amount in soil can greatly improve CEC and soil structure. Mineral soils in the Southeastern USA are generally low in organic matter and contain about 1 to 5% soil organic matter on a volume basis. The warmer, more humid southern climate increases microbial activity leading to the efficient decomposition of organic matter to carbon dioxide, especially where surface tillage is performed. In extreme eastern regions of North Carolina and Virginia, organic soils (Histosols) or mineral soils with histic surfaces are found; these soils may also be found in bay or pocosin areas. In some cases, soil organic matter content exceeds 40% on a volume basis in these soils. These high levels of organic matter developed due to lush vegetation produced in wet soils where limited microbial decomposition occurred under anaerobic conditions. Since extensive drainage is necessary for production agriculture in these soils, loss of organic matter due to oxidation, often referred to as subsidence, is an ongoing process.

The laboratories in the Southeastern US determine soil organic matter by combustion, loss-on-ignition, Walkley-Black, or alkali extraction. The combustion method involves treating soil at high temperature and measuring the carbon dioxide released. The Walkley-Black method involves oxidation of organic C with concurrent reduction of dichromate and analysis of remaining dichromate with ferrous iron. Both of these methods determine the amount of C in soil. Since soil organic matter contains approximately 58% C, a factor is used to estimate the amount of soil organic matter from an analysis of soil C. Loss-on-ignition measures the loss in mass of soil after treating the soil at high temperature to combust and remove organic matter. Alkali extraction of soil was developed by Mehlich and is used in North Carolina. In this method, soil is treated with 0.2 *N* NaOH. The strong alkali dissolves humic and fulvic acids which are major components of soil humus. The dissolved organic C provides a black and brown coloration in a filtrate which is dependent on concentration and is quantified with spectrophotometric analysis.

Details on determining soil organic matter for each of the four methods are described in the following chapters. The section on the combustion method also provides information on N analysis in soil because this method for soil carbon is routinely performed on instruments that also detect total soil N.

*Chapter 5.2*

# Total Carbon and Nitrogen and Organic Carbon via Thermal Combustion Analyses

*T. Provin*

## Application and Principle

Modern soil testing laboratories have quickly adopted the use of automated thermal combustion instrumentation for the determination of carbon (C) and nitrogen (N) in soil and other solid materials. In general, these classes of instrumentation heat the solid sample in a highly concentrated oxygen environment, thereby combusting the reduced carbon compounds and liberating carbon monoxide, carbon dioxide, and nitrogen oxides. Depending on the instrumentation, the gasses are then passed over a series of reducing or oxidizing reagents/catalysts, water and ash separators or gas splitting devices. The method of C or N detection is dependent on instrument manufacturer, element of interest, and concentration of analyte.

The use of modern C and N combustion instrumentation offers significant benefits over older and sometimes inaccurate wet chemistry and ashing methods. Wet chemistry methods, such as Walkley Black and Modified Mebius, utilize a dichromate solution which becomes a hazardous waste. These methods can be extremely slow and may require significant technician time. While the Modified Mebius use of an external heat source helps ensure full organic carbon destruction, the unheated Walkley-Black method must be calibrated for recovery on a regional, sub-regional, or finer basis. This task has commonly been skipped by many laboratories, as it becomes impractical or impossible to address all soils of a region, or insure that various organic amendments and rates are addressed.

Some laboratories have opted to use loss on ignition (LOI) in place of dichromate wet chemistry methods. While LOI does eliminate the disposal costs, this method still requires venting of the muffle furnace and introduces technician safety issues unless the muffle furnace is allowed to significantly cool before removal of the samples. Furthermore, LOI can significantly overestimate organic carbon due to loss of hydration water around ions in soil with significant salt, salts with thermal decomposition temperatures less than the LOI furnace temperature, and hydration water in the interlayers of smectic clays. Research performed by the Texas AgriLife Extension Service Soil, Water and Forage Testing Laboratory found that LOI organic matter determinations in Texas clayey soils were overestimated by 30 to 300%.

Total N wet chemistry methods involve significant sample preparation and chemistry methods. The long used Kjeldahl methods, with the exception of several modified methods that require additional digestion steps, do not recovery oxidized nitrogen forms including nitrate-N and nitrite-N. All Kjeldahl methods require a significant heating source and use concentrated sulfuric acid and a catalyst, commonly selenium. The Kjeldahl method suffers from long digestion time and the disposal costs of the reagents. Unless customized heating apparatus are used, most Kjeldahl methods use block digestors set in fume hoods. The use of fume hoods

increases a laboratory's operating costs due to added air conditioning and heating and hood maintenance costs.

## Equipment and Apparatus

The equipment and apparatus required for automated thermal (Dumas) combustion analyses differ depending on manufacturer and what elements or compounds are analyzed. Numerous instrument companies manufacture systems targeting specific industries or wet chemistry method replacements. Commonly used combustion systems are listed in Table 1.

*Table 1. Various types of combustion units used in analyses of C and N in soil.*

| Type | Furnace | Gases Used † | Elements | Detectors ‡ |
|---|---|---|---|---|
| Medium Temperature | Constant temperature | UHP $O_2$/He | Total N and C | Thermal Conductivity Detector (TCD) for N, TCD or IR for C |
| Medium Temperature | Constant temperature | UHP $O_2$/$CO_2$ | Total N | TCD |
| Induction | Induction | UHP $O_2$/He | Total C | IR |
| Liquid injection | Constant temperature | $CO_2$ free air | Total C and N | Chemical reactive cell or Chemilumisence for N, IR for C |
| Acid treated heated and stirred vessel | NA | $CO_2$ free air, $O_2$, or normal air | Inorganic C | TCD or IR |

† UHP = ultrahigh purity
‡ TCD = thermal conductivity detector, IR = infrared detector

The type of detector utilized on a specific instrument is determined by the elements to be detected and catalysts used. Instrumental analysis techniques for C determinations convert all or part of the C in the sample to $CO_2$ for quantification. For N determinations, all N is converted to either $N_2$ or NO for quantification. Thermal conductivity detectors (TCD) are commonly used to detect $N_2$ or $CO_2$. Nondispersive infrared (NDIR) detectors are used to detect $CO_2$. Chemiluminesence or electrochemical detectors are used for NO.

Each manufacture has designed their systems to operate and perform under specific conditions and matrix types. A review of current instruments on the marketplace will review some level of uniformity with regard to overall system design, however significant proprietary components and software is used to enable the system to have complete analyte recovery. A list of common instrument accessories or additional equipment is given below.

1. Sturdy bench or countertop
2. Dedicated power outlet (maybe 110v or 220v depending on instrument)
3. PC computer
4. High pressure inert gas regulator
5. High pressure oxygen gas regulator
6. High pressure cylinder safety rack
7. Sample racks
8. Analytical balance (0.1 or 0.01 mg accuracy)

## Reagents

Reagents for thermal combustion instruments are often limited to those recommended by the manufacture and will vary depending on analytes determined. In all cases, close attention to the manufacturer's specified gas and reagent quality is required to ensure safe and accurate analysis. A generic list of reagents is included below (Table 2).

*Table 2. List of reagents commonly used in combustion analysis of C and N.*

| Reagent | Analyte |
|---|---|
| Ultrahigh purity oxygen | C and N |
| Ultrahigh purity helium | C and N |
| Tungsten | C and N |
| Copper sticks | N |
| Drying agents | C and N |
| Iron or nickel powder (induction furnaces) | C |
| Sulfuric acid | Inorganic C |
| EDTA or similar organic compound | C and N |
| Sucrose (for high N fertilizers) | N |
| Tin foil | C and N |
| Crucible (instrument specific) | C and N |

## Procedure

Sample Preparation

The sample preparation required that ensures a high level of precision and accuracy in total C and N analysis of soil does not differ from that required in wet chemistry analyses. All soil should be ground to the appropriate fineness to reduce sample heterogeneity. While the requirement for sample grind fineness varies dependant on sample mass combusted, as a general rule, samples should be ground to ensure 100% passage through a 80-100 mesh sieve if less than 0.250 g of sample will be combusted. Even if larger sample sizes are used, the sample should be ground to a minimum fineness to insure 100% passage though a 40 mesh sieve.

## Organic Carbon Methods

Organic carbon analysis can be performed by one of three approaches listed below.

1. Direct measurement of total carbon (TC) and total inorganic carbon (TIC) yielding total organic carbon (TOC) by difference.
2. Chemical removal of the TIC followed by TC determination for TOC.
3. Direct measurement of TOC in the presence of TIC by reduced temperature combustion.

Direct measurement of TC and TIC

Dedicated inorganic C systems are available which interface with C or C/N analyzers and utilize a heated acid treatment converting inorganic C to $CO_2$ which is passed through the instrument detector to determine TIC. The difference between TC determined on a C analyzer and TIC results in TOC.

This method has several advantages including utilization of existing instrumentation, low cost of analysis, and similar techniques as existing wet chemistry methods. An important consideration might require laboratory method modification if soils contain dolomite. Significant alteration in sample heating and gas flush volumes and timing may be required to ensure complete destruction of the dolomite.

Chemical removal of TIC

This method has historically been used to destroy inorganic organic carbon prior to combustion of the soil sample. One reference method developed by the International Organization for Standardization (ISP 10694, 1995), requires acidification for 4 h and then drying for an additional 16 h. The destruction of inorganic carbon becomes increasingly difficult when dolomite is present. Dolomite is fairly acid resistant at room temperature. With warming, the reaction becomes extremely rapid and can potentially cause liquid and solid movement out of the reaction vessel. While laboratories often develop unique approaches toward sample analysis, a pre-acidification method is often used exclusively with crucible loading instruments. Sulfuric acid, sulfurous acid, hydrochloric acid or phosphoric acid is added to the pre-weighed soil previously placed in a ceramic crucible. Following completion of sample fizzing, the crucible is placed in the instrument autosampler. For analyzers which cannot use crucibles, the sample is acidified in a silver foil, dried and then wrapped in tin foil for analysis.

An internal study conducted by the Texas AgriLife Extension Service Soil, Water and Forage Testing Laboratory studied the effectiveness of four different acid types on carbonate destruction and organic carbon recovery (Ali and Provin, 2000). This internal study found the use of either sulfuric and phosphoric acid (4.4 *N*) could result in the loss of up to 85% of highly reactive organic carbon compounds and did measurable destruction of highly resistant carbon. Hydrochloric and nitric acids (4.4 *N*) had less impact on the different carbon pools, but were determined not to be ideal because of elevated potential for instrument corrosion or reagent depletion. If the soil contains dolomite, the soil and acid mixture must be heated to drive the destruction reaction. When heated, dolomite and acid react extremely aggressively and can result in significant loss of soil from the crucibles.

Direct measurement of TOC

The concept of reducing the primary furnace temperature of the instrument to prevent inorganic C destruction has appeared in several journal articles. Research conducted by Texas AgriLife Extension Service Soil, Water and Forage Testing Laboratory (Pitt, 2003) evaluated the recovery of carbon from multiple organic and inorganic carbon sources. A furnace temperature of 650$^{o}$C was determined to be optimum for greater than 98% recovery of organic carbon from most soil and organic samples. A temperature of 675$^{o}$C was required to achieve this recovery for bovine bloodmeal. Additionally, no observable destruction of calcite occurred until furnace temperatures reached 725$^{o}$C.

Several notable modifications and potential errors were noted. First, while all non-furnace conditions were left unchanged from traditional total C and N, selecting an alternative organic C standard for instrument calibration might be required. The L-Glutamine can be replaced by DL-Leucine as an instrument standard since L-Glutamine rapidly charcoals upon introduction into the lower temperature furnace resulting in low C recovery. Additionally, inorganic carbon will be recovered from samples containing minerals with thermal decomposition temperatures below 650 $^{o}$C. The number of carbonate minerals with low thermal decomposition temperatures is limited but does include sodium bicarbonate and sodium carbonate and several iron carbonates.

## Total Carbon and Nitrogen Analysis

The use of automated combustion analyzers for total carbon analysis is relatively straightforward. Outside of normal instrument maintenance and troubleshooting, key concerns are sample homogeneity and sample contamination. While sample fineness was addressed previously, sample contamination can be a considerable source of error. Open autosamplers are subject to air-borne dust particles which can result in elevated C measurements. A common error experienced by some laboratories has been improper marking of crucibles with carbon containing pencil lead or markers. All crucibles should remain free of markings, but be placed in special racks to designate their laboratory identification.

The bulk of total N soil analyses are also straightforward. As with total carbon analyses, proper maintenance is important for precise and accurate results. One problem, although not likely for most surface soils, is low recovery of N in materials with low organic carbon. The recovery of ammonium is aided by the presence of reduced carbon. Thus, samples with extremely low carbon and modest ammonium or urea should be mixed with sucrose or other nitrogen-free carbon compound.

## Troubleshooting

Most troubleshooting on automated combustion instrumentation involves gas flow issues. These flow issues include both gas leaks and gas blockages or flow restrictions. Most manufacturers include onboard diagnostics to detect and pinpoint flow problems. Most blockages are the result of inadequate attention to ash removal or the analyses of high salt containing materials. Careful attention to sample analysis numbers and grouping samples which consume reagents together will enable the technician to better schedule and maintain the instrument.

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*Chapter 5.3*

# Loss on Ignition Method

*H. Zhang and J.J. Wang*

## Application and Principle

This method determines the organic matter (OM) content of soil by oxidizing organic matter to $CO_2$ at an elevated temperature in a muffle furnace and measuring the weight loss. The heating temperature is critical because weight loss can also be affected by loss of water and minerals. An initial temperature of 150$^{o}$C is imposed to ensure complete loss of hygroscopic water in gypsum and to obtain an initial weight without water in the soil (Combs and Nathan, 1998; Schulte and Hopkins, 1996). The soil is exposed to a final temperature of 360$^{o}$C which oxidizes and removes organic matter before obtaining a final weight. The final temperature of 360$^{o}$C is low enough to avoid loss of most carbonates minerals with low thermal decomposition temperatures. This method is a good alternative to the Walkley-Black method which generates hazardous waste and the thermal combustion method which is expensive for instrumentation and supplies.

## Equipment and Apparatus

1. Analytical balance with 0.001 g resolution and 100 g capacity
2. Muffle furnace capable of heating up to 400$^{o}$C
3. Drying oven to heat at 105$^{o}$C
4. Crucibles and rack
5. Desiccator

## Procedure

1. Number crucibles with wax pencil.
2. Weigh crucibles to 3 decimal places. Record this weight as weight 1.
3. Weigh 5 to 10 g of processed soil (dried, < 2mm) into crucibles. This weight does not need recorded.
4. Put crucibles plus samples on a tray in a drying oven set at 105$^{o}$C to remove hygroscopic water. If samples contain gypsum, place crucibles into a muffle furnace set at 150$^{o}$C to ensure water loss.
5. Dry at this temperature for 2 h. It may take some time to reach the intended temperature. Therefore, allow extra time required for oven to warm to intended temperature if it is turned on after placing samples in the oven.
6. Remove crucibles with samples from furnace with tongs, place in desiccator, and allow to cool. Weigh crucibles with samples and record weights to 3 decimal places. Record this weight as weight 2.
7. Place crucibles with samples in a furnace set at 360$^{o}$C.

8. Combust at this temperature for 2 h. Allow time for oven to warm to 360°C to ensure samples are heated at this temperature for a full 2 h.
9. Transfer crucibles with samples from the furnace into a desiccator with tongs and allow to cool.
10. Weigh crucibles with samples and record weights to 3 decimal places. Record this weight as weight 3. Be consistent with sample cooling time after removal from the oven before weighing.

## Calculations

1. OM, % = (weight 2 – weight 3) ÷ (weight 2 – weight 1) × 100

## Analytical Performance

Range and Sensitivity

1. Using an analytical balance with a resolution of 0.001 g results in a sensitivity of 0.02% OM with a 5.000 g sample and 0.01% OM with a 10.000 g sample.
2. The method is best suited for soils with organic matter greater than 1%. In soils with OM less than 1%, errors can occur with weight loss from structural water or thermal decomposition of minerals that can cause significant overestimation of organic matter.

Precision and Accuracy

1. Organic matter determined by LOI is an estimate from an indirect method determined by weight loss of soil upon organic matter removal. Therefore, the accuracy of LOI may not be as good as more direct methods, such as Walkley-Black or thermal combustion, which analyze the carbon content of soil. The estimate of OM from LOI can be regressed to other OM methods on soils from a particular region. The regression equation can be used to determine OM that would be obtained with the direct method. With a combusting temperature of 360°C, the OM regression slope between LOI and the Walkley-Black method ranged from 0.66 to 1.04 and intercept ranged from -0.36 to 0.04 (Combs and Nathan, 1998).
2. Other combusting temperatures have been use such as 400, 450, 500, and 600°C (Nelson and Sommers, 1996; David, 1988; Storer, 1984; Goldin, 1987). Organic matter via LOI at these temperatures was generally correlated well but different from the Walkley-Black method.
3. Gavlak et al. (2003) reports the method is reproducible within ±20%.
4. Results from 151 North American Proficiency Testing samples from 1999 through 2008 show median interlaboratory precision of 8.2% ranging from 4.8 to 21.5% (median absolute deviation/median x 100) with median soil OM of 2.6% ranging from 0.5 to 9.5%.
5. Intralaboratory precision from Oklahoma State University soil testing laboratory on 3 control samples analyzed 15 times ranged from 3.3 to 4.7% (standard deviation/average × 100) for soils with OM ranging from 1.64 to 2.32%.
6. Variability of combustion temperatures with the muffle furnace can cause variability in results. This variability can be accounted for by determining OM concentrations for one soil placed in every position in the muffle furnace. These results can be used to develop correction factors to apply to results based on the position of samples within the oven.

Interferences

1. The initial temperature of 150$^{o}$C is intended to remove water molecules associated with gypsum. This temperature may not be high enough to remove structural waters in the interlayers of smectite minerals. Therefore, this method may not be suitable for soils containing high content of 2:1 layered clay minerals.
2. Temperature above 360$^{o}$C can cause loss of carbonate minerals with low thermal decomposition temperatures. Therefore, a sample of calcium carbonate should be included as a method check to evaluate carbonate loss. If the weight loss of calcium carbonate is greater than 0.05%, the temperature of the muffle furnace needs to be checked to ensure temperature is not exceeding 360$^{o}$C.

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*Chapter 5.4*

# Walkley-Black Method

*R. Mylavarapu*

## Application and Principle

The Walkley-Black (WB) method (Walkley and Black, 1934) determines soil organic carbon (OC) by oxidizing carbon with acidic dichromate ($Cr_2O_7^{2-}$). The oxidation step is followed by titration of excess dichromate with ferrous sulfate. The OC is calculated from the difference between the total dichromate added and the amount of dichromate left unreacted after OC oxidation. The method provides an estimate of soil OM from OC determination with assumptions on the fraction of soil OC reacted and the amount of OC in soil OM. This method was widely considered a standard method for OM after its development but the advent of thermal decomposition instruments have resulted in its limited use. Also, loss on ignition and thermal decomposition methods are more widely used due to their ability to analyze samples in a more time efficient manner.

## Equipment and Apparatus

1. Analytical balance with 0.01 g resolution
2. 250-mL wide mouth graduated Erlenmeyer flasks
3. Repipetter to deliver 10 mL volume
4. Fume Hood
5. Titration stand and burette
6. Stir plate with light
7. Stirring rods
8. Weighing vessel

## Reagents

1. *Potassium dichromate ($K_2Cr_2O_7$), 0.167 M, 1 N*: Dissolve 98.08 g of oven-dried potassium dichromate in approximately 1500 mL of deionized water and dilute to 2 L. After preparation of this solution, transfer to a clean glass bottle for use with a repipetter to deliver 10 mL. Do not mix old potassium dichromate solution with the new solution.
2. *Ferrous sulfate ($FeSO_4 \cdot 7H_2O$), 0.5 M, 0.5 N*: Dissolve 278.02 g of ferrous sulfate in approximately 1500 mL of deionized water. Carefully add 30 mL of concentrated sulfuric acid, mix, cool, and dilute to 2 L. After preparation, this solution may be transferred to a clean 8-L plastic carboy. Do not mix old ferrous sulfate solution with the new solution. The tubing, stopcock, and attachments to the burette should be rinsed three times with new ferrous sulfate solution before titrating any blanks or samples. Prepare a new solution every 30 days.

3. *Ferroine (1,10-Phenanthroline-ferrous sulfate-complex) indicator solution, 0.025 M* : Available from chemical supply companies.
4. *Sulfuric acid ($H_2SO_4$)*: Concentrated and not less than 96%.

## Procedure

1. Titrate blank samples without soil before proceeding with any unknown samples. Add 10 mL of potassium dichromate into a 250 mL Erlenmeyer flask. Titrate with ferrous sulfate. Titrant volume should be approximately 20 mL. Record the exact volume of titrant to the nearest 0.1 mL. If the titrant volume of the two blanks is not within 0.2 mL, clean the burette and associated tubing. Reanalyze two more blanks to determine if the problem has been eliminated.
2. Weigh 1.00 g of processed mineral soil (dried, <2 mm) into a 250-mL wide mouth graduated Erlenmeyer flask. If soil weight is not exactly 1.00 g, record weights for later calculation.
3. Pipet 10 mL of potassium dichromate into each flask and mix carefully by rotating the flask to wet all of the soil.
4. Under a fume hood, carefully add 20 mL of sulfuric acid to each flask and mix gently.
5. Allow flasks to stand for 5 min under the fume hood.
6. Add deionized water to each flask so the final volume is approximately 125 mL. Mix by swirling gently.
7. Allow the samples to cool and return to room temperature. If volume is less than 125 mL after 30 min, add more deionized water to achieve 125 mL volume.
8. Add 5 or 6 drops of ferroine indicator solution and immediately titrate with ferrous sulfate. Use a mixing bar to thoroughly stir the sample as it is titrated. As the titration proceeds, the solution will take on a green color that will change abruptly to reddish-brown when the endpoint of the titration is reached.
9. Record each volumetric reading to the nearest 0.1 mL.

## Calculations

1. Soil organic C (OC) is determined from the meq of $K_2Cr_2O_7$ reacting with the soil to oxidize organic C, which is the difference between total meq of $K_2Cr_2O_7$ added to soil and the meq of $FeSO_4$ that titrated the remaining $K_2Cr_2O_7$ after the reaction.

   $$\text{OC, \%} = (\text{meq } K_2Cr_2O_7 - \text{meq } FeSO_4) \times (0.003 \text{ g C meq}^{-1}) \times \text{cf} \div \text{g soil} \times 100$$

   The oxidation state of C in organic matter is assumed to be 0. The C in organic matter is oxidized to $CO_2$ with an oxidation state of +4. Thus, the equivalent weight of C is 3 g (12 g C ÷ mole × mole C ÷ 4 eq C = 3 g C $eq^{-1}$ = 0.003 g C $meq^{-1}$). An oxidation correction factor (cf) is required since not all the organic C in soil is oxidized with room temperature oxidation. Correction factors can range from 1.14 to 1.32.

2. The meq of $K_2Cr_2O_7$ reacting with soil to oxidize OC is determined from the following equation.

   $$(\text{meq } K_2Cr_2O_7 - \text{meq } FeSO_4) = (mL_B - mL_S) \times 0.5\ N$$

The average volume of ferrous sulfate to titrate two blanks of potassium dichromate without soil is $mL_B$, the volume of ferrous sulfate to titrate the sample is $mL_s$, and 0.5 is the normality of the ferrous sulfate titrant.

Substitution into the equation for OC results in the following.

$OC, \% = (mL_B - mL_S) \times 0.5 \times (0.003 \text{ g C meq}^{-1}) \times cf \div \text{g soil} \times 100$

Using a correction factor (cf) of 1.3 results in:

$OC, \% = (mL_B - mL_S) \times 0.195 \div \text{g soil}$

3. Since there is approximately 58% C in soil organic matter, soil OM can be determined as:

   $OM, \% = (mL_B - mL_S) \times 0.195 \div \text{g soil} \times (100 \text{ g OM} \div 58 \text{ g OC}) =$

   $(mL_B - mL_S) \times 0.336 \div \text{g soil}$

## Analytical Performance

Range and Sensitivity

1. This method is appropriate for mineral soils with OM content less than 6%. Soils with more than 6% OM should be analyzed using a quantity of soil less than 1 g to avoid difficulties in observing the color change at the titration endpoint and exhausting all the potassium dichromate which oxidizes the OM.
2. Each volumetric reading is recorded to the nearest 0.1 mL. This titrant volume change corresponds to 0.03% OM when 1 g of soil is used.

Precision and Accuracy

1. To ensure good precision, the person titrating must be able to view the reddish-brown color change at the endpoint to stop the titration. The aid of a stirring bar and a well lit stirring plate can improve the ability to view the end point.
2. Results from 151 North American Proficiency Testing samples from 1999 through 2008 show median interlaboratory precision of 10.6% ranging from 4.6 to 28.4% (median absolute deviation/median x 100) with median soil OM of 2.0% ranging from 0.5 to 8.1%.
3. Intralaboratory precision from the University of Florida Extension Soil Testing Laboratory on control samples analyzed 15 times averaged 1.09 ± 0.074% (standard deviation/average x 100) for soils with OM ranging from 0.85% to 1.14%.

Interferences

1. Ferrous iron and chloride in soil can result in positive errors in OM since these constituents can result in reduction of $K_2Cr_2O_7$. Ferrous iron should not be a problem in soils thoroughly air-dried since this ensures nearly complete oxidation of ferrous iron to ferric iron. Chloride should not be a concern in well drained soils without a recent fertilizer application. Options for treating soils with high chloride can be found in Nelson and Sommers (1996).

2. Manganese oxide ($MnO_2$) can compete with $K_2Cr_2O_7$ in oxidizing soil OM resulting in negative errors in OM. This is a rare occurrence and only occurs with freshly precipitated $MnO_2$.

## Interpretation

1. Interpretation of OM concentrations regarding soil fertility can be found in Magdoff et al. (1996).

## Effects of Storage

1. Air-dried soil can be stored indefinitely without affecting this measurement.
2. Potassium dichromate and ferrous sulfate solutions should be prepared fresh every 30 d.

## Safety and Disposal

2. The potassium dichromate is defined as a hazardous chemical by the Resource Conservation and Recovery Act due to toxicity (USEPA, 1980a). A laboratory generating more than 100 kg of material in a month is considered a hazardous waste generator that needs to follow hazardous waste disposal protocols defined by the US Environmental Protection Agency (USEPA, 1980b).

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*Chapter 5.5*

# Colorimetric Determination of Humic Matter with 0.2 *N* NaOH Extraction

*D.H. Hardy*

## Application and Principle

Soil organic matter (OM) is composed of plant and animal materials in various stages of degradation. Humus is the relatively stable fraction of OM that is known to be a major contributor to soil's cation exchange capacity (CEC), water holding capacity, and structure. Its abundance gives soils a dark brown to black color.

Organic matter is determined by various methods including loss on ignition (LOI) and chemical methods such as Walkley-Black (1934). Chemically, humus is fractionated into humic and non-humic materials based on solubility in acid or alkali solutions. Humic and fulvic acids are the major components of the humic material. Humic matter (HM) is based on alkali and alcohol extraction of soil organic matter and subsequent determination by absorbance transmission using a colorimeter.

The Soil Testing Lab at the North Carolina Department of Agriculture and Consumer Services determines HM to classify soils for lime requirement. In North Carolina, a significant acreage of Histosols and mineral soils with histic epipedons exist in Northeastern (Tidewater) and Southern regions of the Coastal Plain. Some of these soils are associated with Carolina Bays. Soils are classified according to HM content to determine an appropriate target pH and lime requirement. Mineral soils normally have target pH values above 6.0 to precipitate aluminum and avoid phytotoxic $Al^{3+}$ in soil solution. In soil with high concentrations of OM, $Al^{3+}$ is complexed to the OM which reduces its availability and phytotoxicity to plants (Evans and Kamprath, 1970). Hence, lower pH levels from 5.0 to 5.5 are satisfactory to avoid $Al^{3+}$ toxicity and are used as target pH values for lime recommendations in soils with high humic matter content. The addition of HM with other soil test data can also provide a more comprehensive overview of soil fertility.

## Equipment and Apparatus

1. Soil scoop with 1 $cm^3$ scoop and leveling rod
2. Polystyrene vials with 55-mL capacity (36 x 75 mm or similar size).
3. Syringe pump or pipette to deliver 20 mL
4. Diluter to mix and dispense 5 mL of extract with 35 mL water
5. UV/VIS colorimeter equipped with fiber-optic probe, 2-cm light path, and 650 nm filter. A Brinkman Model 910 colorimeter is used by the Soil Testing Lab at the North Carolina Department of Agriculture and Consumer Services.

## Reagents

1. *Sodium hydroxide (NaOH)*: The alkaline nature of this reagent solubilizes humic acids and $Na^+$ acts as a dispersant.
2. *Pentasodium diethylenetriaiminepentaacetic acid, 40% (w/v) in water (DTPA $Na_5$, $[(NaOOCCH_2)_2NCH_2CH_2]_2NCH_2COONa$)*: This reagent aids in the dispersion of large Ca-humate compounds via complexation of Ca.
3. *Ethanol, denatured ($CH_3CH_2OH$)*: This reagent aids in wetting hydrophobic OM surfaces.
4. *HM extractant*: Following are directions for making 50 L which is enough for 1200 samples. For larger or smaller volumes of the HM extractant, multiply or divide quantities by the appropriate factor. The HM extractant contains 0.2 *M* NaOH, 0.002 *M* DTPA, and 2% ethyl alcohol.
   a. Add 10 L of water to a calibrated 50-L carboy.
   b. Add 400 g NaOH and stir to dissolve.
   c. Add 200 L of DTPA $Na_5$ (40% w/v) and stir.
   d. Dilute to 50 L.
   e. The pH of the extractant should be about 12.

## Procedure

1. Measure 1.0 $cm^3$ of processed soil (dried, <2 mm) into 55-mL polystyrene vials and add 20 mL of extractant with enough force to mix with soil. After 1 h, add another 20 mL of HM extractant with enough force to mix; allow samples to set overnight.
2. Five mL of undisturbed supernatant from each sample is diluted with 35 mL of water. The dilution can occur with a diluter/dispenser by withdrawing 5 mL of supernatant and 35 mL of water before delivery to polystyrene vials with enough force to mix.
3. Using a Brinkman probe fiber-optic colorimeter with a 650-nm filter, set instrument to auto-zero in a solution of 5 mL extractant plus 35 mL of water. Set the instrument to read 100% transmission. Immerse probe into unknown sample and record percent transmission (%T).

## Calculations

1. The humic matter content of a soil is determined by %T (Table 1). The first column is the first digit of %T. The first row is the second digit of %T. The humic matter content is contained within the intersecting cell of the two digits from %T. For example, a transmission value of 75% corresponds to 1.25 g HM (100 $cm^3$ soil)$^{-1}$.

   The data represented in Table 1 can be expressed mathematically as $y = 100.01\ e^{-0.2302x}$ where x and y represent g HM (100 $cm^3$ soil)$^{-1}$ and %T, respectively.

*Table 1. Humic matter (g HM (100 $cm^3$ soil)$^{-1}$) as related to percent transmission (%T) of 5 mL extractant diluted with 35 mL water.*

| | 2nd digit of %T | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1st digit of %T | | | | | | | | | | |
| 1 | 10.00 | 9.59 | 9.21 | 8.86 | 8.54 | 8.21 | 7.96 | 7.70 | 7.45 | 7.21 |
| 2 | 6.99 | 6.78 | 6.58 | 6.38 | 6.20 | 6.02 | 5.85 | 5.69 | 5.53 | 5.38 |
| 3 | 5.23 | 5.09 | 4.95 | 4.81 | 4.69 | 4.56 | 4.44 | 4.32 | 4.20 | 4.09 |
| 4 | 3.98 | 3.87 | 3.77 | 3.67 | 3.57 | 3.47 | 3.37 | 3.28 | 3.19 | 3.10 |
| 5 | 3.01 | 2.92 | 2.84 | 2.76 | 2.68 | 2.60 | 2.52 | 2.44 | 2.37 | 2.29 |
| 6 | 2.22 | 2.15 | 2.08 | 2.00 | 1.94 | 1.87 | 1.80 | 1.74 | 1.67 | 1.61 |
| 7 | 1.55 | 1.49 | 1.43 | 1.37 | 1.31 | 1.25 | 1.19 | 1.14 | 1.08 | 1.02 |
| 8 | 0.97 | 0.92 | 0.86 | 0.81 | 0.76 | 0.71 | 0.66 | 0.60 | 0.56 | 0.51 |
| 9 | 0.46 | 0.41 | 0.36 | 0.32 | 0.27 | 0.22 | 0.18 | 0.13 | 0.08 | 0.04 |

## Analytical Performance

Range and Sensitivity

1. The concentration range that can be analyzed is 0 to 10 g HM (100 $cm^3$ soil)$^{-1}$.

Precision and Accuracy

1. Based on the analysis of three check samples in North Carolina's soil test laboratory, the following data (Table 2) were attained for intralaboratory precision.

*Table 2. Intralaboratory variation from 56 repeated determinations of HM in 3 check soils with measurements taken on different days.*

| Check Soil | %T | | g HM (100 $cm^3$ soil)$^{-1}$ | |
|---|---|---|---|---|
| | mean | standard deviation | mean | standard deviation |
| A | 96.4 | 1.1 | 0.16 | 0.05 |
| E | 12.1 | 1.3 | 9.20 | 0.44 |
| W | 86.1 | 2.0 | 0.65 | 0.10 |

2. Table 1 is based on a calibration conducted long ago using standard humic acid from Aldrich Chemical company (Mehlich, 1984; Donohue, 1992). Table 1 is best considered for qualitative classification of soil according to OM matter content. For more accurate analysis of HM in soil, the procedures in Mehlich (1984) and Donohue (1992) can be consulted for recalibration with use of humic acid extracted from soil or purchased via a chemical supply company.

Interferences

1. Prior to dilution, any suspended material may potentially interfere with light measurements although these are not normally encountered.
2. If dilution occurs with such force that air bubbles are created in the solution to be read, sufficient time to allow air bubbles to diffuse through the samples is required.

## Interpretation

1. North Carolina's soil test laboratory uses HM to determine soil class as mineral (MIN), mineral-organic (M-O), or organic (ORG) as follows.

   MIN: HM $\leq$ 3.37 g (100 $cm^3$ soil)$^{-1}$.
   M-O: HM > 3.37 g (100 $cm^3$ soil)$^{-1}$ and HM $\leq$ 5.23 g (100 $cm^3$ soil)$^{-1}$
   ORG: HM > 5.23 g (100 $cm^3$ soil)$^{-1}$

   Soils in portions of North Carolina's Coastal Plain region, and to a more limited extent in the Mountain region, contain considerable amounts of organic matter that limits $Al^{3+}$ solubility and phytotoxicity which allows lower than normal soil pH to be tolerated for crop production. Consequently, the class system is utilized to set target pH for which lime rate is determined. Target pH values are set at 5.0, 5.5, and 6.0 to 6.5 for ORG, M-O and MIN soil classes, respectively. The pH range given for the MIN soils is dependent upon the crop to be grown. For example, corn has a target pH of 6.0 whereas alfalfa has a target pH of 6.5.

   A measure of HM along with other soil test data such as CEC allows for inferences to be made of other soil test properties such as texture. The more comprehensive overview of soil properties can help with nutrient management decisions.

## Effects of Storage

1. Air-dry soil can be stored indefinitely without affecting this measurement. The HM extraction solution keeps indefinitely as long as it is covered from outside air.

## Safety and Disposal

1. When making and dispensing the extractant, safety glasses, full face shield, gloves, and lab coat are required due to the caustic nature of the basic solution. Some laboratories may require neutralization of the soil and extract before discarding into the sink due to high pH.

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*Unit 6*

# Soil Characterization

*Chapter 6.1*

# Introduction to Soil Characterization

*D.E. Kissel*

Methods for cation exchange capacity (CEC), particle size analysis, and salinity are presented in the following chapters. Cation exchange capacity and particle size distribution are intrinsic properties of soil that change very little over time under normal circumstances. Soil salinity, on the other hand, changes more readily under varying soil management practices. These three soil characteristics affect soil fertility.

Cation exchange capacity is a measure of the negative charges on a soil's exchange sites that can retain and exchange positively charged ions (cations) by electrostatic attraction. Cation exchange capacity can be determined by direct measurement or by estimation based on quantities of calcium, magnesium, potassium, and exchangeable acidity from a routine soil fertility test. Laboratories at Auburn University and Mississippi State University test soils with a wide range of CEC and provide nutrient recommendations for phosphorus (P) and K based on CEC (Adams et al., 1994; Hsu, 1979). Another use of CEC measurement occurs with land application of biosolids containing heavy metals where the quantity of metal that can be land-applied is limited by the CEC of the soil (Epstein, 2003).

Soil is a mixture of minerals, organic matter, water and air. The solid particles, minerals and organic matter, constitute about 50% of the soil by volume. Soil particle size distribution defines soil texture and affects water and air movement, nutrient holding capacity, pore sizes and root growth. Soil particle size and organic matter have an important influence on plant available water (Ritchie et al., 1999). Along with climate, plant available water has a significant influence on crop yield potential.

All soils contain soluble salts with major dissolved inorganic ions of $Na^+$, $Mg^{2+}$, $Ca^{2+}$, $K^+$, $Cl^-$, $SO_4^{2-}$, $HCO_3^-$, and $CO_3^{2-}$. Soils are considered saline when they contain high levels of soluble salts. This condition can be detrimental to crops with a lowering of the osmotic potential, which reduces the ability of the plant to extract soil water, or presence of high concentrations of ions that can be toxic such as $H_2BO_3^-$ and $Ba^{2+}$. In arid and semiarid climates, soluble salts accumulate in the soil surface due to high evapotranspiration along with insufficient water to leach soluble salts from the soil. For irrigated cropland, high salt levels can accumulate with poor soil drainage and irrigation water containing elevated levels of soluble salts. In addition, salt-affected soils can be caused by salt water spills from oil drilling activities as well as high rates of manure and sludge applications. In coastal areas, seawater intrusion has also become an increasingly important cause of soil salinity. Sodic soils are those soils containing high levels of $Na^+$ relative to other major cations on the soil's exchange sites. High $Na^+$ content results in dispersion of soil particles and poor water permeability.

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*Chapter 6.2*

# Cation Exchange Capacity

*F.J. Sikora, K.K. Crouse, S. Heckendorn, G. Huluka, C.C. Mitchell, K.P. Moore and J.L. Oldham*

## Application and Principle

Cation exchange capacity (CEC) is an intrinsic property of soil defining the concentration of negatively charged sites on soil colloids that can adsorb exchangeable cations. Cation exchange capacity can be a good indicator of soil productivity and is useful for making recommendations of phosphorus (P), potassium (K), and magnesium (Mg) if testing soils of different textures. Cation exchange capacity is also used for regulatory purposes in monitoring land application of biosolids.

Cation exchange capacity is a measure of exchangeable bases and soil acidity at some specific soil pH. The exchangeable bases and acidity neutralize negative charges arising from permanent charges due to isomorphic substitution in clays, or pH-dependent charges from hydroxyl groups on clay and oxides or carboxyl groups on soil organic matter. A common method for determining CEC uses 1 *M* ammonium acetate at pH 7 (neutral $NH_4OAc$) and is a standard method used for soil surveys by the Natural Resource Conservation Service (Burt, 2004; Hendershot et al., 2008; Sumner and Miller, 1996). An advantage of CEC measured at a constant pH of 7 is elimination of CEC variability due to differences in soil pH. Thus, comparisons of CEC can occur across varied soil types and lime applications. A disadvantage of the neutral $NH_4OAc$ method is that it may not provide a realistic depiction of the actual CEC at the natural pH of the soil, particularly with soils having considerable pH-dependent charge and a soil pH that is significantly different from 7. An unbuffered salt extract can be used to determine CEC at the natural pH of soil (Burt, 2004; Sumner and Miller, 1996; Pansu and Gautheyrou, 2006). Summation of equivalent charge concentrations of basic cations (Calcium (Ca), Mg, K, and sodium (Na)) and acidic cations (aluminum (Al), Iron (Fe), and Manganese (Mn)) extracted with an unbuffered salt is referred to as effective CEC.

Cation exchange capacity can be determined by neutral $NH_4OAc$, or by indirect estimation from routine soil test results. Routine soil analysis to assess soil fertility commonly includes Ca, Mg, K, and Na analysis in the soil extract. The concentrations of bases extracted are similar to concentrations of exchangeable bases. Acidity at pH 7 can be estimated from a soil-buffer pH routinely used to assess lime requirement. The sum of equivalent charges from exchangeable bases and acidity from routine analysis provides a value for CEC that is similar to CEC from the neutral $NH_4OAc$ method. Two problems with this method of estimating CEC occurs with the presence of soluble salts from fertilizers or solubilization of calcium or magnesium carbonates. Bases extracted from soluble salts or carbonates are not exchangeable bases and thus will result in false high estimates for CEC.

Cation exchange capacity is not a major criterion used when making soil fertilizer recommendations. Fertilizer recommendations are usually calibrated for soils with a similar soil texture where different recommendations based on CEC are not required. There are exceptions for laboratories testing soils with a wide range of textures that adjust nutrient recommendations

based on CEC. Land grant universities in the Southeaster US that adjust P or K recommendations based on CEC are Auburn University (Adams et al., 1994) and Mississippi State University (Hsu, 1979).

This chapter provides details on determining CEC and base saturation with neutral $NH_4OAc$. Also, formulas are presented for using routine results from soil fertility analysis to estimate neutral $NH_4OAc$ CEC and base saturation.

## CEC and base saturation with neutral $NH_4OAc$

### Equipment and Apparatus

1. Analytical balance with 0.01 g resolution
2. 250-mL beakers
3. 7.0-cm Buchner funnels
4. Filter paper (7-cm Whatman #1 or #42)
5. 250-mL suction flask connected to vacuum pump
6. 250-mL volumetric flasks
7. Balance, stir plate, stir bars and container for reagents
8. Instrumentation for $NH_4^+$ analysis. Instrumentation for $Ca^{2+}$, $Mg^{2+}$, $K^+$, and $Na^+$ analyses.

### Reagents

1. *1 M $NH_4OAc$ at pH 7.00*: Following are directions for making 10 L of this reagent. Multiply quantities by appropriate values for making larger or smaller volumes. Make the solution in a fume hood to avoid breathing vapors of ammonia and acetic acid.
   a. Add 580 mL of concentrated glacial acetic acid ($CH_3COOH$) to approximately 5 L of water.
   b. Add 680 mL of concentrated ammonium hydroxide ($NH_4OH$, 29% $NH_3$ w/w).
   c. Add water to yield a volume of approximately 1900 mL.
   d. Adjust pH to 7.00 with dropwise additions of either ammonium hydroxide or acetic acid.
   e. Dilute to 10 L.
   f. Thoroughly stir contents to ensure complete mixing.
2. *Ethanol, 95% w/w ($CH_3CH_2OH$)*
3. *1 M KCl*: Following are directions for making 10 L of this reagent. Multiply quantities by appropriate values for making larger or smaller volumes.
   a. Add approximately 8 L of water to a carboy calibrated with a volume of 10 L.
   b. Add 745 g KCl and stir to dissolve.
   c. Dilute to 10 L after dissolution is complete.
   d. Thoroughly stir contents to ensure complete mixing.

### Procedures

1. Weigh 10 g of processed mineral soil (dried, <2 mm) into a 250-mL beaker.
2. Add 25 mL of 1 *M* $NH_4OAc$ to the soil. Refrain from mixing the beaker in a circular fashion to avoid soil wicking onto the sides of the beaker. Cover and let set overnight.

3. For each sample, prepare a 7-cm Buchner funnel by fitting it with a 7-cm Whatman #42 filter paper. Wet the filter with a minimum amount of 1 *M* $NH_4OAc$. Insert the funnel into a 250-mL suction flask. Turn on vacuum pump to seat the moistened filter. Stir and transfer the soil-$NH_4OAc$ mixture into the filter.
4. Measure approximately 75 mL $NH_4OAc$ for each sample into a plastic squirt bottle with one bottle for each sample. Use about 10 mL of the $NH_4OAc$ in the bottle to transfer all of the soil to the Buchner funnel.
5. Cover the soil with a 7.0-cm Whatman #1 filter paper to keep the soil moist between leachings.
6. Leach the soil 5 to 7 times with 10 to 15 mL increments of $NH_4OAc$. Do not let the soil dry between leachings.
7. Transfer the leachate to a 250-mL volumetric flask and bring to volume with 1 *M* $NH_4OAc$. Analyze the solution for Ca, Mg, K, and Na using atomic absorption spectrophotometry, flame emission spectrophotometry, or inductively coupled plasma spectrophotometry.
8. To remove excess $NH_4OAc$ in the soil, leach the soil with ethanol. Leach the soil with about 25 mL portions of ethanol five to six times for a total volume of about 150 mL. Discard the leachate.
9. To remove adsorbed $NH_4$ in the soil, leach the soil with 1 *M* KCl. Leach the soil with about 25 mL portions of 1 *M* KCl four to five times for a total volume of about 125 mL.
10. Transfer the leachate to a 250-mL volumetric flask and bring to volume using 1 *M* KCl. Analyze the solution for $NH_4$ concentration using colorimetry, distillation, or ion-selective electrode potentiometry.

## Calculations

1. If mg $L^{-1}$ of $NH_4$-N is quantified in the leachate, use the following to calculate CEC.

   CEC ($cmol_c\ kg^{-1}$) = CEC (meq $(100\ g)^{-1}$)
   (mg $NH_4$-N $L^{-1}$) × (0.25 L ÷ 10 g soil) × (1 meq $NH_4$-N ÷ 14 mg $NH_4$-N) × 100 =
   (mg $NH_4$-N $L^{-1}$) × 0.179

   If mg $L^{-1}$ of $NH_4$ is quantified in the leachate rather than $NH_4$-N, use 18 mg $NH_4$ instead of 14 mg $NH_4$-N.

2. Calculate exchangeable bases (Ca, Mg, K, and Na) as follows.

   Ca ($cmol_c\ kg^{-1}$) = Ca (meq $(100\ g)^{-1}$) =
   (mg Ca $L^{-1}$) × (0.25 L ÷ 10 g soil) × (2 meq Ca ÷ 40.1 mg Ca) × 100 =
   (mg Ca $L^{-1}$) × 0.125

   Mg ($cmol_c\ kg^{-1}$) = Mg (meq $(100\ g)^{-1}$) =
   (mg Mg $L^{-1}$) × (0.25 L ÷ 10 g soil) × (2 meq Mg ÷ 24.3 mg Mg) × 100 =
   (mg Mg $L^{-1}$) × 0.206

K ($cmol_c\ kg^{-1}$) = K ($meq\ (100\ g)^{-1}$) =
(mg K $L^{-1}$) × (0.25 L ÷ 10 g soil) × (1 meq K ÷ 39.1 mg K) × 100 =
(mg K $L^{-1}$) × 0.0639

Na ($cmol_c\ kg^{-1}$) = Na ($meq\ (100\ g)^{-1}$) =
(mg Na $L^{-1}$) × (0.25 L ÷ 10 g soil) × (1 meq Na ÷ 23.0 mg Na) × 100 =
(mg Na $L^{-1}$) × 0.109

3. Calculate exchangeable acidity as follows.

   Acidity ($cmol_c\ kg^{-1}$) = CEC – (Ca + Mg + K + Na)

4. Calculate percent base saturation as follows.

   Base saturation (%) = (Ca + Mg + K + Na) ÷ CEC × 100

5. The Soil Science Society of America accepts $cmol_c\ kg^{-1}$ as the unit for CEC, exchangeable bases, and exchangeable acidity (ASA, CSSA, SSSA, 1998). Another unit used for these values is $meq\ (100\ g)^{-1}$. Units of $cmol_c\ kg^{-1}$ and $meq\ (100\ g)^{-1}$ are interchangeable since 1 $meq\ (100\ g)^{-1}$ = 1 $cmol_c\ kg^{-1}$.

## Analytical Performance

Range and Sensitivity

1. The range and sensitivity for determining concentrations of $NH_4$, Ca, Mg, K, and Na depend on the analytical procedures chosen for analysis. Consult methodology details of the analytical technique used to assess range and sensitivity.

Precision and Accuracy

1. Typical measurements of intralaboratory precision for CEC, Ca, Mg, K, and Na measured on one quality control sample used at the University of Kentucky soil test laboratory are shown below. An $NH_3$ ion-selective electrode was used for $NH_4$ analysis and an ICP was used for base cation analysis (Table 1).

*Table 1. Intralaboratory variations for CEC and exchangeable bases of a quality control sample analyzed 10 times on separate days.*

| Parameter | Number of measurements | Mean | Standard deviation |
|---|---|---|---|
| | | -------- $cmol_c\ kg^{-1}$-------- | |
| CEC | 10 | 15.7 | 1.30 |
| Ca | 10 | 8.00 | 0.20 |
| Mg | 10 | 3.77 | 0.11 |
| K | 10 | 0.46 | 0.04 |
| Na | 10 | 0.05 | 0.01 |

Interferences

1. Cation exchange capacity can be overestimated if not all of the excess $NH_4$ is leached out of the sample with ethanol or it can be underestimated if some of the adsorbed $NH_4$ is displaced during washing.
2. Exchangeable Ca can be overestimated if free calcium carbonate or gypsum exists since Ca leached from soil with $NH_4OAc$ addition can be from dissolution of these minerals in addition to exchangeable Ca. Presence of calcium carbonate is more likely when soil-water pH is greater than 7.0. Overestimation of exchangeable Ca results in an overestimation of base saturation.
3. Cation exchange capacity can be underestimated if calcium carbonate or gypsum exists. When soil colloids are saturated with $NH_4$ from $NH_4OAc$, Ca can neutralize some of the negative charge on the soil colloids resulting in incomplete saturation of exchange sites with $NH_4$. Calcium carbonate is more likely to exist when soil-water pH is greater than 7.0.
4. Exchangeable Na is usually low in most of the soils of the Southeastern US. Any filter paper used containing leachable Na may cause an overestimation of exchangeable Na. A control can be run to test for background levels of Na and other bases. If significant levels of Na and bases exist, try different filter papers or wash the filter paper with $NH_4OAc$. To wash the filter paper, place a single filter into a Buchner funnel. Leach the filter with ammonium acetate, and let dry. This usually takes about 10 min. After the filter has dried, place the filter in a box designated for rinsed filters.

## Interpretation

1. Soil texture has an important impact on CEC since negatively charged colloids dominate in the clay-sized fraction. In general, sandy loams have CEC less than 10 cmol $(kg)^{-1}$, loams and silt loams have CEC between 10 and 20 cmol $(kg)^{-1}$, and clay loams have CEC greater than 20 cmol $(kg)^{-1}$. Organic matter content also has a strong influence on soil CEC since organic colloids have a greater CEC compared to clay minerals.
2. The CEC has limited use for fertilizer recommendations since P and K recommendations have normally been calibrated for soils in a particular region with a similar CEC. However, there are exceptions where soils of wide variability are tested. Phosphorus, K, and Mg recommendations are based on CEC in Alabama (Auburn University). Potassium and Mg recommendations are based on CEC in Mississippi (Mississippi State University). Information on nutrient recommendations based on CEC in these states is provided in the section entitled “Estimated CEC”.
3. Cation exchange capacity is used as a basis for recommendations on regulated application of biosolids as presented in Epstein (2003).

## Effects of Storage

1. Air-dried soils may be stored several months without affecting CEC or base saturation measurements.

## Safety and disposal

1. The chemicals used in this procedure should be stored and disposed of according to routine laboratory procedures.
2. Prepare the 1 *M* $NH_4OAc$ solution in a fume hood to avoid breathing ammonia and acetic acid vapors.

# Estimated CEC

Cation exchange capacity and base saturation determined from neutral $NH_4OAc$ can be estimated from routine soil test results to assess soil fertility. An advantage of using this method is no additional testing is required beyond obtaining routine soil fertility test results. A disadvantage occurs with errors from summation of extractable cations and acidity estimated from soil test results rather than direct measurement via single ion displacement after saturating exchange sites. Also, erroneously high CEC values can be calculated by the release of nonexchangeable base cations which can occur with high soluble salt levels or dissolution of carbonates and gypsum.

A summary of the methods used to obtain estimated CEC by soil test laboratories in the Southeastern US are reported below. The estimated CEC is a summation of bases obtained from an extraction used to assess nutrient levels plus an estimate of acidity from a soil-buffer pH measurement. The acidity determined from soil-buffer pH is an estimate of the acidity neutralized when soil pH is raised to 7. This acidity includes exchangeable acidity, as $Al^{3+}$, and residual acidity, as $H^+$ released from hydroxyl and carboxyl groups on soil solids (SSSA, 1996). Therefore, the calculated CEC methods reported below provide an estimate for CEC determined with neutral $NH_4OAc$. This CEC is different than effective CEC which is determined as a summation of charges from exchangeable base cations and $Al^{3+}$ displaced with an unbuffered salt solution (Sumner and Miller, 1996). The effective CEC is a measure of soil's negatively charged sites adsorbing exchangeable cations at the native pH of the soil and is lower than CEC using neutral $NH_4OAc$.

## Exchangeable Bases

Exchangeable bases (Ca, Mg, K, and Na) determined with neutral $NH_4OAc$ are approximately the same as bases extracted with Mehlich-1, Mehlich-3, or Lancaster extractants. The following equations are used to convert from lbs $A^{-1}$ to $cmol_c\ kg^{-1}$.

Exchangeable Ca ($cmol_c\ kg^{-1}$) = Extractable Ca (lbs $A^{-1}$) ÷ 400
Exchangeable Mg ($cmol_c\ kg^{-1}$) = Extractable Mg (lbs $A^{-1}$) ÷ 240
Exchangeable K ($cmol_c\ kg^{-1}$) = Extractable K (lbs $A^{-1}$) ÷ 780
Exchangeable Na ($cmol_c\ kg^{-1}$) = Extractable Na (lbs $A^{-1}$) ÷ 460

If bases are reported in units of mg $kg^{-1}$ (ppm), the following equations are used.

Exchangeable Ca ($cmol_c\ kg^{-1}$) = Extractable Ca (mg $kg^{-1}$) ÷ 200
Exchangeable Mg ($cmol_c\ kg^{-1}$) = Extractable Mg (mg $kg^{-1}$) ÷ 120
Exchangeable K ($cmol_c\ kg^{-1}$) = Extractable K (mg $kg^{-1}$) ÷ 390
Exchangeable Na ($cmol_c\ kg^{-1}$) = Extractable Na (mg $kg^{-1}$) ÷ 230

The percent base saturation is determined from the summation of the exchangeable cations divided by the estimated CEC times 100.

Calcium, Mg, K, and Na are considered for CEC determination in South Carolina. Other states that calculated CEC only use Ca, Mg, and K. Omission of Na is not considered to cause too great an error because its concentration is usually very low in soils of the Southeastern US.

The calculation of exchangeable bases from extractable bases is best conducted for acid soils. At soil pH values greater than 7, there is a greater likelihood of overestimating exchangeable bases by extracting nonexchangeable Ca and Mg from carbonate minerals.

## Alabama

The Soil Testing Laboratory at Auburn University estimates CEC by summation of Mehlich-1 extractable K, Ca, Mg, and acidity estimated from the modified Adams-Evans buffer (Huluka, 2005). Acidity is determined from the modified Adams-Evans buffer pH according to:

Acidity ($cmol_c\ kg^{-1}$) = 8 × (8 – modified Adams-Evans soil-buffer pH).

The $cmol_c\ kg^{-1}$ of Ca, Mg, K, and acidity are summed to determine CEC and is referred to as summation CEC (CEC+).

A relationship was obtained between soil-water pH and the fraction of the CEC occupied by acidic cations ($Hsat_1$), or base unsaturation, by analyzing several Alabama soils (Huluka, 2005) as shown below.

Soil-water pH = $7.60 - 5.71 \times (Hsat_1) + 2.99 \times (Hsat_1)^2$

The relationship can be used to determine $Hsat_1$ at any soil-water pH value using a quadratic equation. Acidity from modified Adams-Evans soil-buffer pH and $Hsat_1$ can be used to calculate cation exchange capacity as shown below.

CEC = (acidity from modified Adams-Evans soil-buffer pH)/ $Hsat_1$

This value is referred to as the buffer CEC since it is calculated from soil-water pH and soil-buffer pH. The CEC+ and buffer CEC have very similar values for normal soils (Hue and Evans, 1983). Any discrepancy between these two values may be due to the presence of high soluble salts, calcareous soils, free limestone, or abnormal soil pH. Discrepancies may also occur from errors in analysis of cations, soil-water pH, or soil-buffer pH. For quality control purposes, both CEC values can be generated from routine laboratory data and criteria can be set

to alert technicians when large differences in these values occur. Samples can be reanalyzed to ensure differences are not due to analytical error.

The CEC+ results are used to separate soils into 4 groups as shown below.

Group 1. Estimated CEC < 4.7 $cmol_c\ kg^{-1}$
Group 2. Estimated CEC = 4.7 to 9.0 $cmol_c\ kg^{-1}$
Group 3. Estimated CEC > 9 $cmol_c\ kg^{-1}$
Group 4. Estimated CEC > 9 $cmol_c\ kg^{-1}$ in the Black Belt region of Alabama.

Phosphorus, K, and Mg recommendations are based on the CEC categories defined above. For P, the CEC-based soil groups are used to identify those clayey soils with a high P fixation capacity at higher CEC (> 9 $cmol_c\ kg^{-1}$). These soils have a different calibration for crop response to P compared to soils with lower CEC (≤ 9 $cmol_c\ kg^{-1}$) soils. Soils in group 4 are tested using the Lancaster extractant for nutrient analysis. Each soil CEC group has been shown to have different calibration curves for crop response to K. Group 1 soils have a different calibration curve for crop response to Mg compared to the finer-textured soils of groups 2 through 4. Phosphorus, K, and Mg recommendations based on soil test values and CEC are presented in Chapter 1.2 and Adams et al. (1994).

## Kentucky

At the University of Kentucky soil test laboratory, CEC is calculated and reported on soil test reports by summing Mehlich-3 extractable bases (Ca, Mg, and K) and acidity from Sikora-2 soil-buffer pH. Acidity is determined with the following equation.

Acidity ($cmol_c\ kg^{-1}$) = 8 × (7.5 – Sikora-2 soil-buffer pH )

## Mississippi

The Mississippi State soil testing laboratory calculates CEC for soil test reports from routine soil analysis using the Lancaster extractant by summing the extracted bases (Ca, K, and Mg) with acidity estimated from soil-buffer pH using the Woodruff buffer. Acidity is determined with the following equation.

Acidity ($cmol_c\ kg^{-1}$) = 10 × (7.20 – modified Woodruff soil-buffer pH) × 10

Estimated CEC is used to provide fertilizer recommendations for K and Mg. Mississippi research has found that higher soil test K levels are needed to maximize yields of crops in clay loam and clay soils compared to coarser textured soils (Hsu, 1979). Magnesium availability in Mississippi is considered a function of both pH and CEC, thus recommendations are based upon these two factors. Potassium and Mg recommendations based on soil test values and CEC are presented in Chapter 1.2.

## South Carolina

At the Clemson University soil test laboratory, CEC is calculated and reported on soil test reports from routine soil analysis by summing Mehlich-1 extractable bases (Ca, Mg, K, and Na) and acidity from the Moore-Sikora buffer (Sikora and Moore, 2008). Acidity is determined from the following equation.

Acidity ($cmol_c\ kg^{-1}$) = 8 × (8.00 – Moore-Sikora soil-buffer pH)

## Virginia

At the Virginia Tech Soil Testing Laboratory, CEC is calculated and reported on soil test reports by summing Mehlich-1 extracted bases (Ca, Mg, and K) and acidity from the Mehlich buffer. Acidity is determined from the Mehlich buffer pH according to the following equation.

Acidity ($cmol_c\ kg^{-1}$) = 37.94 – (5.928 × Mehlich soil-buffer pH)

The equation was developed from a comparison of soil acidity neutralized in NaOH titrations of soil to pH 6.5 with Mehlich soil-buffer pH on typical Virginia soils.

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*Chapter 6.3*

# Particle Size Determination by Hydrometer Method

*G. Huluka and R. Miller*

## Application and Principle

Soil texture is a basic property of soil that affects soil physical properties and management. Under normal conditions, it is considered a permanent property of a soil. Soil texture affects soil water and nutrient holding capacities, water and air movements, pore sizes and plant root growth. Because of these important roles, soil texture is considered a master soil variable.

Soil is a mixture of minerals, organic matter, water and air of which minerals and organic matter constitute about 50% of the soil by volume. Soil solids are made up particles of different sizes. Soil texture is defined by relative proportions of sand, silt, and clay. The United States Department of Agriculture (U.S. Salinity Laboratory Staff, 1954) defines sand, silt, and clay as particles with sizes from 2 to 0.05 mm, 0.05 to 0.002 mm, and less than 0.002 mm, respectively.

Soil particle size is determined using Stoke's law which predicts the velocity of free falling spherical soil particles in water based on particle size. The larger the particle size, the faster the settling velocity. The viscosity of water affects particle settling velocity and is itself affected by temperature. Therefore, a correction is necessary for temperatures deviating from a standard temperature of 20$^{o}$C. Particle size analysis is accomplished first by the addition of a chemical dispersant, sodium metaphosphate, with subsequent mechanical agitation. With the hydrometer method, density of the soil suspension is determined using a Bouyoucos hydrometer at specific times depending on the particle size being measured (Gee and Bauder, 1986). With the pipette method, clay particles are removed with a pipette at a specific time, sand particles are separated with a 270 mesh (53.3 µM) screen, and both the clay and sand particles are quantified with gravimetric measurement (Gee and Bauder, 1986). The method described here is for particle size analysis using the hydrometer method.

The chemical dispersant may not completely disperse soil particles in the presence of soil constituents such as organic matter, carbonate minerals, soluble salts, and some oxides. These soil constituents coagulate particles via various forces that are stronger than the force of dispersion provided by the dispersant. Thus, the removal of organic matter, carbonates, soluble salts, and oxides may be necessary depending on the concentration of the components and the accuracy required. These soil constituents can be removed by pretreating the soil. Pretreatment options are presented in this method. A more detailed presentation of pretreatment options are provided by Gee and Bauder (1986), Kroetsch and Wang (2008), and American Society for Testing and Materials (2008).

## Equipment and Apparatus

1. Analytical balance with 0.01 g resolution
2. ASTM Soils Hydrometer 152H with Bouyoucos scale in g $L^{-1}$ (American Society for Testing and Materials, 2008)
3. Electrical stirrer with 10,000-rpm motor
4. Stainless steel blender cup (600 to 850 mL)

5. Sedimentation cylinder marked at 1-L volume with 40 cm space above the mark
6. Plunger and rubber stopper for 1-L cylinder
7. Timer
8. Thermometer
9. Centrifuge capable of 1500 rpm with 250-mL glass centrifuge bottles if soil is pretreated

## Reagents

Pretreatment to remove carbonates, organic matter, oxides, or salt

1. *Hydrochloric acid (HCl)*
2. *Hydrogen peroxide ($H_2O_2$), 30% w/w*
3. *Sodium hydrosulfite ($Na_2S_2O_3$)*
4. *Citrate-bicarbonate buffer*: Prepare 0.3 M sodium citrate ($Na_3C_6H_5O_7$) (88.4 g $L^{-1}$) and add 125 mL of 1 *M* sodium bicarbonate ($NaHCO_3$) (84 g $L^{-1}$) to each liter of citrate solution.

Particle size analysis

1. *Amyl alcohol*
2. *Dispersing agent*: Following are directions for making 1 L. Multiply quantities by the appropriate factor for making larger volumes.
   a. Add about 500 mL of deionized water to a 1-L volumetric flask.
   b. Add 7.93 g of sodium carbonate ($Na_2CO_3$) and stir to dissolve.
   c. Add 35.7 g of sodium metaphosphate $(NaPO_3)_6 \cdot Na_2O$ and stir to dissolve.
   d. Dilute solution to 1 L and mix well.

## Procedure

With pretreatment to remove carbonates, organic matter, oxides, or salt

1. Prepare soil by air drying and pulverizing to pass a 10 mesh sieve (< 2 mm). Weigh 50 ± 0.05 g of fine-textured soil (i.e., soil loam, silt loam, or clay loam) or 100 ± 0.05 g of coarse-textured (i.e., sand, loamy sand, or sandy loam) into a 300-mL beaker.
2. Removal of carbonates: Add 50 mL deionized water and sufficient 1 *M* HCl to reduce the soil pH to between 3.0 and 4.0. Stir and allow sample to set for 10 min until there is no effervescence.
3. Removal of organic matter: Add 10 mL of hydrogen peroxide to the beaker. When frothing subsides heat to 90°C and add additional 10 mL portions of hydrogen peroxide until frothing subsides. Rinse down walls of beaker and continue heating for about an hour to ensure excess hydrogen peroxide is consumed.
4. Removal of iron oxides and soluble salts: Transfer the sample to a 250-mL glass centrifuge bottle. Add 150 mL of citrate-bicarbonate buffer to the sample in the centrifuge bottle. Add 3 g of sodium hydrosulfite gradually as samples may froth. Place in water bath at 80°C and stir intermittently for 20 min. Centrifuge for 10 min at 1500 rpm and decant the centrifugate. Add 150 mL of deionized water to the sample and mix well. Centrifuge for 10 min at 1500 rpm and decant the centrifugate. Repeat the centrifugation process with added deionized water until the centrifugate is clear.
5. Removal of soluble salts without iron oxide removal: Transfer the sample treated with hydrogen peroxide to remove organic matter to a 250-mL glass centrifuge

bottle. Add 100 mL water and centrifuge for 10 min at 1500 rpm and decant centrifugate. Repeat the centrifugation process with additional water until supernatant is clear.

6. One can apply a single removal procedure, all the removal procedures, or some combination of removal procedures. After the final removal procedure employed, the sample is transferred to a blender cup for use with an electrical mixer. Continue with step 2 in the following procedural outline for no pretreatment.

Without pretreatment

1. Prepare soil by air drying and pulverizing to pass a 10 mesh sieve (< 2 mm). Weigh 50 ± 0.05 g of fine-textured soil (i.e., soil loam, silt loam, or clay loam) or 100 ± 0.05 g of coarse-textured (i.e., sand, loamy sand, or sandy loam) into a 300-mL beaker.
2. Add deionized water approximately within 10 cm of the rim.
3. Add 50 mL of dispensing agent and let it soak for 20 min.
4. Place the blender cup on electrical mixer and stir for 5 min.
5. Transfer the soil suspension to 1 L measuring cylinder. Use additional water to transfer all soil if necessary.
7. Fill to the 1 L mark with deionized water.
8. Use a plunger with up and down stroke at least five times to mix sediments from the bottom of the cylinder.
9. Immediately place a stopper on the top of the cylinder and mix it by turning upside down at least five times.
10. Place it on a counter table and immediately start a timer, and remove the stopper. If the suspension is covered with foam, add three drops of amyl alcohol.
11. Slowly lower a dry hydrometer into the suspension.
12. Record the hydrometer level at the solution surface at exactly 40 s.
13. Measure the temperature of the suspension.
14. Remove the hydrometer and place it in deionized water and dry with towel before reuse.
15. Repeat steps 2 through 14 with a duplicate sample. The first hydrometer reading ($R_{1st}$) is the average of the hydrometer levels at 40 s from the duplicate samples.
16. Record two hydrometer levels after 8 h duplicates of the same sample. Make sure not to mix the sample. Remove the hydrometer after each reading, place it in deionized water, and dry with towel before reuse. The average of the hydrometer levels at 8 h from the duplicate samples is the second hydrometer reading ($R_{2nd}$).
17. Measure the temperature of the suspension.
18. Include a blank without soil following the same steps as for the sample. Hydrometer level readings from the blank are designated as $R_{C1}$ for the 40 s reading and $R_{C2}$ for the 8 h reading.

## Calculations

1. The first Bouyoucos reading in g $L^{-1}$ provides the content of clay and silt in 1 L of solution. The percentage of clay and silt in the sample is subtracted from 100 to obtain percent sand.

$$\% \text{ Sand} = 100 - ((R_{1ST} - R_{C1}) / \text{sample weight} \times 100)$$

2. The second Bouyoucos reading in g $L^{-1}$ provides the content of clay in the 1 L solution. Percent clay in the sample is determined by the following equation.

   % Clay = ($R_{2ND}$ – $R_{C2}$) / sample weight × 100

3. Percent silt in the sample is determined by the following equation.

   % Silt = 100 – % Sand – % Clay

4. For temperature correction, add or subtract 0.36 g $L^{-1}$ for each degree above or below 20°C, respectively.

## Analytical Performance

Range and Sensitivity

1. The method has a detection limit of 2.0 % for sand, silt, and clay.

Precision and Accuracy

1. The method is reproducible to within ± 8%.

Interferences

1. Organic matter, carbonates, soluble salts and iron oxides can interfere if they are at a significant concentration and not removed from the sample. Their presence usually results in an overestimation of silt content and underestimation of clay content since they act to cement clay particles together which settles from solution like larger silt particles. If organic matter is greater than 3.5 % or carbonates are greater than 2%, soil should be pretreated to remove these constituents for more accurate analysis.
2. The theoretical time for the 2nd reading is 7.72 h, but durations as low as 2 h (Bouyoucos, 1962), and 6 h (Ashworth, et al., 2001) have been suggested depending on the required accuracy, dominant soil texture, and type and amount of interferences.

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*Chapter 6.4*

# Measurement of Soil Salinity and Sodicity

*J.J. Wang, T. Provin, and H. Zhang*

## Application and Principle

Soil salinity is defined as the soluble salt content in soil solution. Soil salinity is measured by electrical conductivity (EC) or total dissolved solids (TDS) in soil solution. The two are closely related but EC is the measurement most often made. There are several different approaches to measure soil EC. An appropriate procedure may be selected depending on the type of information needed in a particular situation. When a rapid in-situ measurement is desired, non-contacting terrain EC sensors including electromagnetic (EM) systems may be used (Rhoades, 1990). Such a procedure has often been used for characterizing salinity of large fields, and it generally has lower accuracy than EC measurements of water extracts of soil performed in a laboratory. The determination of EC in soil-water extracts is accomplished using a conductivity cell which measures the conductance of electricity through the solution between two metal plates. Alternatively, TDS can be determined by a more labor intensive procedure based on residue weight after evaporation to dryness after filtration (Rhoades, 1996). This chapter will focus on common laboratory procedures for characterizing soil salinity based on EC measurement.

Laboratory procedures for measuring EC of soil are based on water extracts from soil. Extraction based on a water-saturated paste of soil serves as a standard procedure because it is the lowest water volume that can be added to soil with sufficient volume extracted for analysis and it provides a close simulation of soil solution content under most field conditions. For these reasons, plant tolerance to salinity has been based on EC values of a saturation paste extract (US Salinity Laboratory Staff, 1954; Mass and Hoffman, 1977). Other soil/water extract ratios such as 1:1 and 1:2 have also been adopted by many laboratories because the methods are less labor intensive than the saturated paste method.

Soil sodicity is defined as the sodium saturation of cation exchange capacity sites in the soil (CEC) and is termed the exchangeable sodium percentage (ESP). Although exchangeable bases can be used to determine soil sodicity, a more common and less laborious method for assessing soil sodicity is to determine the sodium adsorption ratio (SAR) which is the ratio of $Na^{+}$ to $Ca^{2+}$ and $Mg^{2+}$ in soil solution or water extracts. There is a close relationship between SAR of saturated paste and ESP that can be used to determine ESP from SAR measurements.

## Equipment and Apparatus

<u>Saturated paste extraction</u>

1. Analytical balance with 0.1 g resolution
2. 250-mL plastic or glass beakers
3. Buchner funnel and Erlenmeyer flasks for vacuum extraction
4. Vacuum pump or line
5. Filter paper (Whatman No. 2 or equivalent)
6. Reciprocating shaker

7. Conductivity cell
8. Conductivity meter, preferably with temperature compensation
9. Inductively couple plasma-atomic emission spectrometry (ICP-AES), ion chromatography (IC), or flow injection analysis (FIA) if individual solutes are determined

Soil/water extraction

1. Analytical balance with 0.01 g resolution
2. 125-mL plastic or glass Erlenmeyer flasks
3. Automatic solution dispenser
4. Filter funnels and vials for receiving filtrates
5. Filter paper (Whatman No. 2 or equivalent)
6. Reciprocating shaker
7. Conductivity cell
8. Conductivity meter, preferably with temperature compensation
9. Inductively couple plasma-atomic emission spectrometry (ICP-AES), ion chromatography (IC), or flow injection analysis (FIA) if individual solutes are determined

1:1 or 1:2 soil/water EC measurement without extraction

1. Analytical balance with 0.01 g resolution
2. 125-mL plastic or glass Erlenmeyer flasks
3. Automatic solution dispenser
4. Reciprocating shaker
5. Conductivity cell
6. Conductivity meter, preferably with temperature compensation

## Reagents

1. *Potassium chloride (KCl), 0.01 M*: Dissolve 0.7456 g KCl in 1 L of deionized water. The EC for this solution is 0.147 dS $m^{-1}$ at 25°C.
2. *Potassium chloride (KCl), 0.1 M*: Dissolve 7.456 g KCl in 1 L of deionized water. The EC for this solution is 12.9 dS $m^{-1}$ at 25°C.

## Procedure

Saturated paste extraction

1. Weigh 100 to 250 g field-moist or processed (air dried, <2 mm) soil into a 250-mL plastic or glass beaker.
2. Add deionized water to the soil while slowly stirring it using a spatula. At saturation, the soil paste should glisten as it reflects the light. The soil paste will flow slightly when the container is tipped and slides freely and clearly off the spatula.
3. After mixing, allow the sample to stand for at least 1 hr and then recheck for saturation. If the paste stiffens or loses its glisten, add more water and remix. If free water collects on the surface after standing, add more soil and remix. Allow the mixed sample to equilibrate for a total of 18 h or overnight.
4. Transfer the saturated paste to a Buchner funnel with Whatman No. 2 filter paper set on an Erlenmeyer flask designed for vacuum filtration. Apply vacuum to collect filtrate. Refilter if the initial filtrate is cloudy.
5. Save the filtrate for EC and solute analyses.

6. Soils high in clay content have considerably higher water holding capacity and therefore the laboratory may elect to extract a smaller mass of soil. Vacuum extraction of high clay content soils require significantly longer extraction times.
7. Limit vacuum tension to avoid evaporating filtrate from the suction flasks.
8. For laboratories conducting numerous saturated paste samples from high clay soils, the use of syringe vacuum extraction units allow unattended operation and eliminates dehydration of filtrates by vacuum pumps. A filter press using compressed air is more efficient than vacuum in obtaining extracts from saturated paste or suspensions with wider soil/water ratios (Fann Instrument Company, 2013).

1:1 or 1:2 soil/water extraction

1. Measure 20 g (± 0.05 g) or 20 $cm^3$ of processed soil (dried, <2mm) into a 125-mL glass or plastic Erlenmeyer flask.
2. Add 20 or 40 mL of deionized water to the flask to make a soil/water ratio of 1:1 or 1:2 depending on the extraction ratio of preference.
3. Shake extraction flask on reciprocating mechanical shaker for 1 hr.
4. Filter suspension immediately and collect the extract in plastic containers. Refilter if the filtrate is cloudy.
5. Save the filtrate for EC and solute analyses.
6. Larger soil sample size with the same soil/water ratio may be used to generate a greater volume of extract for other analyses such as determining individual ionic solutes.

1:1 or 1:2 soil/water EC measurement without extraction

1. Measure 20 g (± 0.05 g) or 20 $cm^3$ of processed soil (dried, <2mm) into a 125-mL beaker or similar container.
2. Add 20 or 40 mL of deionized water to the soil to make a soil/water ratio of 1:1 or 1:2 depending on the extraction ratio of preference. Stir soil and water with glass or Teflon-coated rod to mix the slurry well.
3. Repeat stirring two additional times during the next 30 min.
4. Allow suspension to settle and measure the soil pH and electrical conductivity in the supernatant.
5. This alternative method is quicker than the filtration method but has several limitations including: 1) tube shaped dipping conductivity electrodes are of limited value due to time required to clean and potentially foul the electrode, 2) the presence of sparingly soluble salts and minerals (e.g., gypsum) may not dissolve during the 30 min, thus skewing the EC measurement to a lower value, and 3) extremely low buffered soils (CEC<3) may precipitate $Ca^{2+}$ as $CaCO_3$ from dissolved $CO_2$ in an open system and thus result in a lower value.
6. This quicker method can be performed in conjunction with soil pH measurement of soil slurries and is an excellent screening tool that laboratories can routinely conduct to provide information on when to conduct more exhaustive soil salinity tests.

Analysis of EC with Conductivity Meter

1. Electrical conductivity measured with a conductivity cell and meter is expressed as conductance of the solution in siemen (S) multiplied by a cell constant defining the geometry of the metal plates in the conductivity cell. The cell constant is the length (L) between metal plates divided by

the cross-sectional area (A) of the metal plates (L/A = $cm^{-1}$). A conductivity cell should be selected with a cell constant that is optimal for expected EC values to be measured (Table 1).

*Table 1. Optimum cell constants for various EC ranges.*

| EC Range<br>dS $m^{-1}$ | Cell Constant<br>$cm^{-1}$ |
|---|---|
| 0.0005 to 0.4 | 0.1 |
| 0.01 to 2 | 1 |
| 1 to 200 | 10 |

2. Calibrate the conductivity meter using 0.010 *M* KCl solution following manufacturer's recommendations in the operation and calibration of the conductivity meter. This solution should yield EC of 1.412 dS $m^{-1}$ at 25°C. A 0.1 *M* KCl solution should be used to calibrate the meter when measuring high salinity soils. The EC of 0.1 *M* KCl is 12.89 dS $m^{-1}$ at 25°C. In most meters, the standard EC calibrated measurement is set with respect to a cell constant of 1 $cm^{-1}$ regardless of the actual cell constant of the conductivity cell.
3. Place the calibrated conductivity cell in the extract making sure the metal plates are fully immersed in solution and make the EC measurement.

Analysis of specific solutes with ICP-AES

1. Calibrate the ICP-AE spectrophotometer using multiple element standards following manufacturer's recommendations in the operation and calibration of the instrument.
2. Obtain a portion of a soil/water extract for individual solute analysis. Dilute the sample if concentrations are above the highest calibration standard. While most inorganic solutes in extracts can be determined by ICP-AES, some ICP-AE spectrophotometers are unable to analyze chloride and other anions. Therefore, ion chromatography or flow injection analysis can be used for these analytes.

## Calculations

1. Soil EC
   Soil EC is reported in the unit of dS $m^{-1}$. This is the accepted SI unit from the International System of Units. Another commonly reported unit for EC is mmho $cm^{-1}$. These units are equivalent to one another.

2. Total dissolved solids (TDS)
   Soil salinity may be expressed as TDS which can be calculated based on EC measurement using the following empirical equation.

   TDS, mg $L^{-1}$ or ppm = CF × EC

The conductivity factor (CF) is 640 for EC in dS $m^{-1}$. The CF could vary from 550 to 900 depending on the solutes. The CF is generally high in chloride-rich solution and low in sulfate-rich solution.

3. Cations and anions
   Cations and anions determined in the solution extract can be converted to concentrations in soil using the following equation.

   Solute in soil, mg $kg^{-1}$ = (mg $L^{-1}$ in extract) × N

   The value for N is 1 for 1:1 soil/water extraction ratio and 2 for 1:2 soil/water extraction ratio. This calculation cannot be used for saturated paste extracts due to the inconsistent ratio of soil to water in preparing the paste. However, if the amount of air-dried soil and water used to make the paste are known, the calculation could be performed with N = (mL of water) / (grams of air-dried soil).

4. Sodium adsorption ratio (SAR)
   Sodium adsorption ratio can be calculated according to the following equation.

   $SAR = Na^{+} \div (Ca^{2+} + Mg^{2+})^{1/2}$

   Values for $Na^{+}$, $Ca^{2+}$, and $Mg^{2+}$ represent the mM concentration of the ions in a saturated paste extract. Some laboratories convert ion concentrations from 1:1 or 1:2 soil/water extractions to the saturated paste equivalent concentrations to determining SAR (US Salinity Laboratory Staff, 1954; Zhang et al., 2005).

5. Conversion of SAR to exchangeable sodium percentage (ESP)
   Soil sodicity is defined by the ESP of soil. Several Western US soils were used to develop a relationship between ESP and SAR (Evangelou, 1998). The empirical equation developed from these soils with SAR from saturated paste ranging from 0 to 60 (mmol $L^{-1})^{1/2}$ is shown below.

   ESP = (-1.26 + 1.475 × SAR) ÷ (0.9874 + 0.01475 × SAR)

   This relationship is based on the Gapon equation by assuming a Gapon constant for Na and (Ca+Mg) exchange as 0.0147 (mmol $L^{-1})^{-1/2}$ (Evangelou, 1998).

## Analytical Performance

Range and Sensitivity

1. The range of typical EC values depends on the soil location. Mineral soils in the humid regions of the Southeastern US are typically less than 1 dS $m^{-1}$. Soils in the dry climatic regions of the Southeastern US can range up to 200 dS $m^{-1}$.
2. Consult Table 1 for the appropriate cell constant to use for the range of EC measurements to be made.
3. For calculation of SAR, an ICP-AE spectrophotometer may be used to determine individual ions of an extract. Modern ICP-AE spectrophotometers can reliably detect metal elements in

water extracts of soils to 0.1 mg $L^{-1}$ for major elements and 0.01 mg $L^{-1}$ for trace elements, which are satisfactory for making soil and water salinity evaluations.

Precision and Accuracy

1. Laboratory measurements of EC for soil and water salinity are generally accurate and reproducible down to 0.005 dS $m^{-1}$.
2. Results from 151 North American Proficiency Testing samples from 1999 through 2008 provides interlaboratory precision for EC in saturated pastes, 1:1 soil/water extracts, and 1:2 soil/water extracts (Table 2).

*Table 2. Values for EC and respective interlaboratry precision on 151 samples tested in the North American Proficiency Testing program from 1999 through 2008.*

| | EC | | Precision† | |
|---|---|---|---|---|
| | Median | Range | Median | Range |
| | ---- dS $m^{-1}$ ---- | | ---- % ---- | |
| Saturated paste | 0.68 | 0.21-10.7 | 12.7 | 5.5-34.8 |
| 1:1 soil/water extract | 0.30 | 0.07-3.87 | 17.9 | 5.8-45.5 |
| 1:2 soil/water extract | 0.19 | 0.04-2.78 | 16.7 | 7.5-40.0 |

† Interlaboratory precision determined as median absolute deviation ÷ median × 100 for each sample tested at several laboratories. Number of laboratories participating ranged from 23 to 51.

Interferences

1. Common errors in EC measurement are often due to inadequate sample circulation and electrode fouling. A four electrode cell may be used to overcome the problems of electrode fouling associated with the conventional two electrode cells.
2. Exposure of an extract sample to air may cause changes in EC due to the dissolution of atmospheric gases such as $CO_2$. The latter is significant when the extract sample contains little dissolved solids.
3. Calcium carbonate can precipitate in the extract resulting in low EC measurements. When EC measurement is not immediately performed after obtaining an extract, 1 drop of 0.1% sodium hexametaphosphate solution may be added per 25 mL of the extract to prevent the precipitation of $CaCO_3$.
4. Common pipet-type and dip-type conductivity cells will serve the purpose of most EC measurements of soil extracts. Flow-through conductivity cells may be used for EC measurements expected below 0.01 dS $m^{-1}$ to minimize exposure of measurements to the atmosphere which can affect EC.
5. Soil EC may be determined by direct measurement in soil suspensions when individual solutes are not required. However, these measurements were found to be generally lower than those made in extracts of similar soil/water ratios (Hogg and Henry, 1984).

## Interpretation

1. The methods outlined here are convenient ways of assessing soil salinity with measurement of EC and soil sodicity with measurement of exchangeable $Na^{+}$, $Ca^{2+}$, and $Mg^{2+}$ and determination of ESP. Definitions of saline and sodic soils are based on analysis of saturated paste extracts and a general guideline for effects on plant growth is provided in Figure 1. While 1:1 and 1:2 soil/water ratios for determining soil salinity are commonly performed, the interpretations of soil salinity and sodicity as related to crop response are more accurately based on measurements made in saturated pastes. Therefore, the conversion of EC or SAR in soil-water extracts to that of a saturated paste extract is necessary. Electrical conductivity in soil-water extracts of 1:1 and 1:2 soil/water ratios will be lower than EC of saturated paste extracts due to dilution in the wider soil-water ratios. Electrical conductivity and individual ions in 1:1 or 1:2 soil/water ratio extracts can be correlated with saturated paste extracts but the correlations are different for different soils (Hogg and Henry, 1984; Zhang et al., 2005). Therefore, caution needs to be exercised in the interpretation of soil salinity and sodicity from extraction of 1:1 and 1:2 soil/water ratios under local soil-climate-crop conditions.

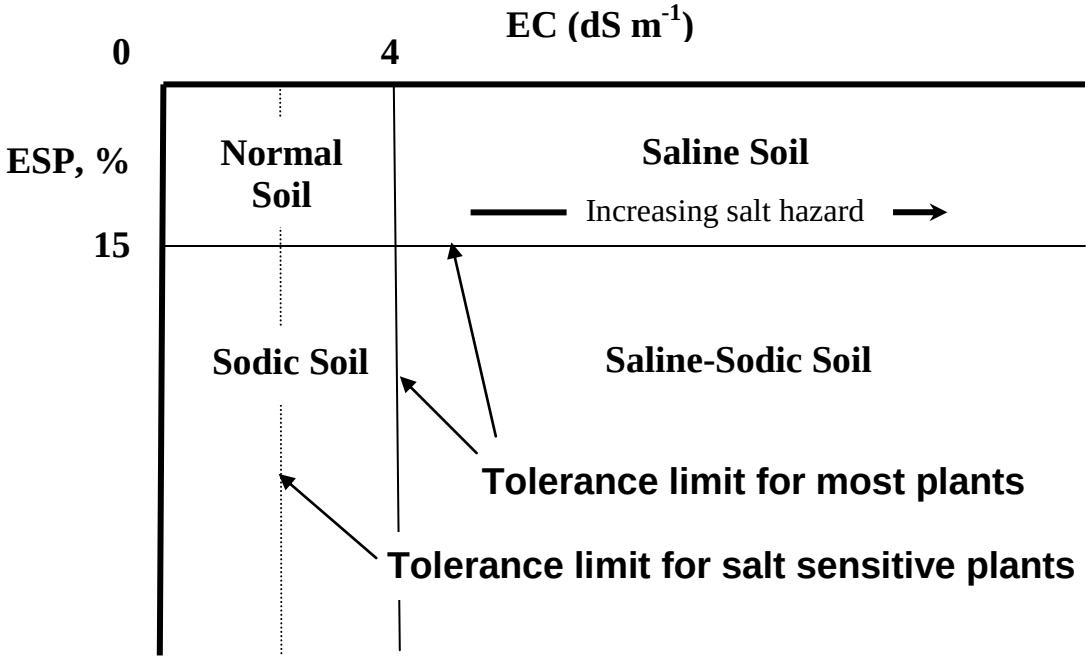


*Fig. 1. Definition of saline and sodic soil and general guidelines for crop response from measurements of EC (dS $m^{-1}$) and ESP (%) of saturated paste extracts.*

2. A guideline for defining soil salinity based on EC of 1:1 and 1:2 soil/water extract ratios is provided in Tables 4 and 5. These are general guidelines only since a more accurate assessment of soil salinity is derived from EC measurement of saturated paste extracts.

*Table 4. Electrical conductivity measurements in 1:1 soil/water extracts and their relation to soil salinity (Gartley, 2011).*

| EC (dS $m^{-1}$) | Degree of Salinity |
|---|---|
| 0 – 0.39 | Non-saline |
| 0.40 – 0.80 | Very Slightly Saline |
| 0.81 – 1.20 | Slightly Saline |
| 1.21 – 1.60 | Moderately Saline |
| 1.61 – 3.20 | Strongly Saline |
| >3.20 | Very Strongly Saline |

*Table 5. Electrical conductivity measurements in 1:2 soil/water extracts and their relation to soil salinity (Gartley, 2011).*

| EC (dS $m^{-1}$) | Degree of Salinity | EC (dS $m^{-1}$) | Degree of Salinity |
|---|---|---|---|
| Coarse sand to loamy sand | | Loamy fine sand to loam | |
| 0 – 1.1 | Non-saline | 0 – 1.2 | Non-saline |
| 1.2 – 2.4 | Slightly Saline | 1.3 – 2.4 | Slightly Saline |
| 2.5 – 4.4 | Moderately Saline | 2.5 – 4.7 | Moderately Saline |
| 4.5 – 8.9 | Strongly Saline | 4.8 – 9.4 | Strongly Saline |
| >= 9.0 | Very Strongly Saline | >= 9.4 | Very Strongly Saline |
| Silt loam to clay loam | | Silty clay loam to clay | |
| 0 – 1.3 | Non-saline | 0 – 1.4 | Non-saline |
| 1.4 – 2.5 | Slightly Saline | 1.5 – 2.8 | Slightly Saline |
| 2.6 – 5.0 | Moderately Saline | 2.9 – 5.7 | Moderately Saline |
| 5.1 – 10.0 | Strongly Saline | 5.8 – 11.4 | Strongly Saline |
| >= 10.0 | Very Strongly Saline | >= 11.5 | Very Strongly Saline |

## Effects of Storage

1. Air-dried soils may be stored several months without affecting the EC and SAR measurement.

## Safety and disposal

1. The chemicals used in this procedure pose no safety risk and therefore can be stored and disposed of according to routine laboratory procedures.

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# *Unit 7*

# Other Tests

*Chapter 7.1*

# Test Procedures for Greenhouse Root Media

*D.D. Warncke*

## Application and Principle

Root media used for production of plants in greenhouses and nurseries are composed of light-weight, natural and processed material such as peat, perlite, vermiculite, sand, bark, coconut fiber (coir), compost and similar materials. These are mixed together in various combinations to provide the actual root media. These lightweight mixes are easy to handle and provide good aeration and moisture-holding properties, but provide limited nutrient-holding capability. Natural soil systems provide plant available nutrients in the soil solution (intensity factor) and have a reserve nutrient supplying capacity with exchangeable cations, "fixed" nutrients and insoluble complexes (capacity factor). Most prepared root media contain only small amounts of soil, if any, and therefore have a limited nutrient reserve (capacity factor). Hence, the availability of nutrients in most prepared root media is dependent on the intensity factor.

Geraldson (1957) developed an "intensity and balance" testing system for poorly buffered sandy soil systems using the saturation extraction approach adopted by the U.S. Salinity Laboratory Staff (1954). Saturation extracts of greenhouse root media provide reliable measures of plant available nutrients (Lucas and Rieke, 1968; Lucas et al., 1972). Researchers in the Netherlands (Sonneveld and van den Ende, 1971; Sonneveld et al., 1974) found saturation extract results to be a dependable measure of the available nutrient status of peat-based mixes. In summarizing test results over a 2-year period, Whipker et al. (1994) demonstrated that root media analysis by saturation extraction is a valuable tool for evaluating greenhouse nutrition problems.

Saturation extraction methodology provides several advantages over previous procedures (Warncke, 1975, 1976, 1986). For many years, soil test laboratories have handled prepared soilless root media samples in a manner similar to that for field soil samples. Analytical procedures were modifications of the Spurway test procedures (Spurway and Lawton, 1949). These worked well for testing greenhouse soil mixes when soil was the base material. However, as the composition of greenhouse root media has changed to include peat and processed materials, the field soil testing procedures have become inadequate. The major shortcomings in treating greenhouse root media in the same way as field soils are related to handling and diagnostic sample size. Drying, grinding and sieving greenhouse media samples results in significant alteration of the sample properties. Trying to measure out a uniform, small sample (2.0 or 1.7 $cm^3$) from a heterogeneous mix of materials is difficult. In addition, interpretation of the results must take into account bulk density which may range from 0.2 to 1.2 g $cm^{-3}$. The Saturated Media Extract (SME) method has been shown to successfully eliminate these handling, sampling and interpretation problems (Warncke, 1975, 1976, 1986). This procedure can also be used to evaluate the suitability of composts for use in growing plants.

With the SME method, a large sample of the root media (400 $cm^3$) is extracted and analyzed which reduces sampling error. With no preliminary handling necessary, samples can be processed and analyzed quickly. The water holding characteristics of the various root media

tend to be related to the bulk density. This acts as an automatic compensator for differences in bulk densities which affect interpretation of results from saturation extracts. As demonstrated by Geraldson (1970), nutrient balance is very important in weakly buffered systems as exists with greenhouse root media. With the SME method, nutrient balance information is readily calculated. Root media which contain slow-release fertilizer can be extracted by the saturation extract method with very little inflation of the test results (Warncke, 1979). With other handling and extraction procedures, test values are greatly inflated due to excessive solubilization of the slow-release fertilizer.

Available micronutrients levels in plant growth media are important for the growth of container grown plants. Micronutrients include zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu). In peat and bark based root media, the basic micronutrients are complexed by organic compounds (Verloo, 1980). Hence, the concentrations of these micronutrients in a water saturation extract are quite low. Zinc and Mn concentrations rarely exceed 0.8 mg $L^{-1}$ and Fe rarely exceeds 4.0 mg $L^{-1}$. Therefore, it is difficult to distinguish between deficient and adequate levels. In evaluating 15 extractants, Berghage et al. (1987) found extractable levels of Fe, Mn, and Zn could be increased greatly by using weak solutions of various salts, acids or chelates in the saturating solution with the saturation extract procedure. Saturation with a 0.005 *M* DTPA solution was found to most consistently increase extractable micronutrient levels while having only a minor effect on other key test parameters such as soluble salts, nitrate, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and chloride (Cl).

## Equipment and Apparatus

1. 600-mL plastic beaker
2. Spatula
3. Buchner funnel, 11-cm
4. Filter paper (Whatman No. 1) 11-cm
5. Vacuum flask, 500-mL
6. Vacuum pump
7. Vial, snap-cap 100-mL
8. Conductivity meter, preferably with temperature compensation
9. Conductivity cell with cell constant of 1.0 $cm^{-1}$
10. Thermometer
11. pH meter
12. pH glass electrode with an internal reference element or a separate reference electrode
13. Ammonium, nitrate and chloride electrodes with internal reference elements or with separate reference electrodes
14. UV-VIS spectrophotometer
15. Flame emission spectrometry, atomic absorption spectrometry, or inductively couple plasma-atomic emission spectrometry (ICP-AES)
16. Volumetric flasks and pipettes as required for preparation of reagents and standard solutions

## Reagents

1. *Distilled or deionized water*
2. *Potassium chloride (KCl), 0.01 M*: Following are direction for making 1 L. Multiply or

divide the amounts by the appropriate factor for making other quantities.
   a. Add about 800 mL of water to a 1 L volumetric flask.
   b. Add 0.7456 g KCL to the flask and stir to dissolve.
   c. Dilute to 1 L and mix well.
3. *DTPA, 0.005 M*: This reagent is used instead of water to extract micronutrients in the modified saturation media extract method. Following are directions for making 1 L. Multiply or divide the amounts by the appropriate factor for making other quantities.
   a. Add about 800 mL of water to a 1 L volumetric flask.
   b. Add 1.97 g diethylenetriaminepentaacetic acid (DTPA, $[(HOOCCH_2)_2NCH_2CH_2]_2$ $NCH_2COOH$) to the flask and stir to dissolve.
   c. Heating the water to 50°C and stirring facilitates dissolution of the DTPA. After the solution has cooled, make to volume with deionized water.
4. Reagents for determining pH, nitrate-nitrogen ($NO_3$-N), P, K, Ca, Mg, and micronutrients of interest.

## Procedure

Saturated media extract with water

1. The saturated media extract method was developed at Michigan State University and has been routinely used in their soil test laboratory. It allows extraction of moist samples just as they come from greenhouses. Drying of samples is unnecessary and undesirable. Fill a 600-mL beaker about two-thirds full with the root medium. Gradually add deionized water while mixing until the sample is just saturated. At saturation the sample will flow slightly when the container is tipped and is easy to work with a spatula. After mixing, allow the sample to equilibrate for 1 h and then recheck the criteria for saturation. The saturated sample should have no appreciable free water on the surface, nor should it have stiffened. Adjust as necessary by addition of root medium or deionized water. Allow the mixture to equilibrate for an additional 30 min.
2. Determine the pH of the saturated sample by carefully inserting the electrodes. Shake the electrodes gently to attain good solution contact.
3. Attach a Buchner funnel lined with filter paper to a vacuum flask. Apply a vacuum and transfer the saturated sample into the Buchner funnel. Spread the sample out with a spatula and tap the funnel to eliminate entrapped air and to insure good contact between the saturated sample and the filter. Continue vacuum, collecting the extract in the flask. No more than 15 min of vacuum should be required. Transfer the extract to a snap-cap vial. All subsequent analyses are done on the extracted solution.
4. *Soluble Salts*: Use 0.01 *M* KCl to calibrate the conductivity meter with temperature compensation. A 0.01 *M* KCl solution should have an electrical conductivity of 1.412 dS $m^{-1}$ at 25 °C. Another common unit used is mmho $cm^{-1}$ which is equivalent to dS $m^{-1}$. Rinse the conductivity cell and dip into the extract solution making sure metal plates of the cell are immersed in solution. Record electrical conductivity in dS $m^{-1}$. If extract volume is limited, the conductivity cell can be inverted and extract poured into the cell.
5. *Nitrate-N ($NO_3$-N), ammonium-nitrogen ($NH_4$-N), and chloride (Cl)*: Nitrate and ammonium can be determined with the appropriate specific ion electrode or by cadmium reduction (nitrate) and Nesslerization (ammonium) through an autoanalyzer unit. With rapid conversion of ammonium to nitrate via nitrification, analysis of just nitrate is usually

adequate. Use of nitrate specific electrode is preferred due to the ease of analysis and wide concentration ranges usually present. Determine the millivolt reading with a nitrate electrode and specific ion meter and obtain the concentration of nitrate from a standard curve. Chloride can be determined with a chloride ion-selective electrode and specific ion meter or with ICP-AES.

6. *P, K, Ca, Mg, and Na*: Determine P on an aliquot of the extract with an accepted colorimetric procedure or with ICP-AES. Determine K, Ca, Mg, and Na on an aliquot of the extract by flame emission, atomic absorption spectroscopy, or with ICP-AES.
7. *Micronutrients*: Micronutrients can be determined with atomic absorption spectrometry or ICP-AES.

Saturated media extract method with DTPA

1. By using 0.005 *M* DTPA as the primary saturating solution, extraction of the basic micronutrients (Zn, Mn, Fe, and Cu) can be greatly enhanced. Some indication of boron (B) availability may also be determined. The saturated media extract method with DTPA involves a change in the procedure used to saturate the growth media and in the measurement of pH.
2. Place 400 $cm^3$ of growth media into a 600-mL beaker.
3. Add 100 mL of 0.005 *M* DTPA.
4. While mixing, gradually add deionized water to bring the media just to the point of saturation. From this point on, proceed as indicated under the saturated media extract method with water, except for pH determination. Since the DTPA solution affects the media pH, use one part of the media by volume and two parts of deionized water by volume for a separate determination of the media pH.

## Calculations

1. Soluble salt levels are determined by the electrical conductivity measurement in dS $m^{-1}$. The electrical conductivity can theoretically be converted to total dissolved solids in mg $L^{-1}$ with multiplying electrical conductivity by 640. However, an empirical factor of 700 provides more practical information. Results for nitrate-N, P, K, Ca, Mg, Na, and Cl are reported as mg $L^{-1}$ of extract. Nutrient balance is determined by calculating the percent of nutrient as a portion of total salt concentration using the following equations.

   a. Total soluble salt concentration (mg $L^{-1}$) = electrical conductivity (dS $m^{-1}$) × 700

   b. Nutrient as percent of total salt concentration (%) =
   (nutrient, mg $L^{-1}$) ÷ (total soluble salt concentration, mg $L^{-1}$) × (100)

## Interpretation

1. The suitable pH, soluble salt and nutrient levels vary with the greenhouse or nursery crop being grown and management practices. Table 1 can be used for interpreting results obtained with either the water or DTPA saturation extracts. When using DTPA in the extraction, the generally adequate ranges for key micronutrients are 0.7 to 2.5 mg $L^{-1}$ for B, 0.5 to 1.5

mg $L^{-1}$ for Cu, 15 to 40 mg $L^{-1}$ for Fe, 5 to 30 mg $L^{-1}$ for Mn, and 5 to 30 mg $L^{-1}$ for Zn. The specific satisfactory levels vary with the plants grown.

*Table 1. General interpretation guidelines for greenhouse growth media analyzed by the saturated media extract method with water or DTPA.*

| Analysis | Low | Acceptable | Optimum | High | Very High |
|---|---|---|---|---|---|
| Electrical conductivity, dS $m^{-1}$ | 0-0.75 | 0.75-2.0 | 2.0-3.5 | 3.5-5 | 5.0+ |
| $NO_3$-N, mg $L^{-1}$ | 0-39 | 40-99 | 100-199 | 200-299 | 300+ |
| P, mg $L^{-1}$ | 0-2 | 3-5 | 6-10 | 11-18 | 19+ |
| K, mg $L^{-1}$ | 0-59 | 60-149 | 150-249 | 250-349 | 350+ |
| Ca, mg $L^{-1}$ | 0-79 | 80-199 | 200+ | - | - |
| Mg, mg $L^{-1}$ | 0-29 | 30-69 | 70+ | - | - |

2. Desired percentages of nutrients in relation to total soluble salts are 8 to 10% $NO_3$-N, less than 3% $NH_4$-N, 11 to 13% K, 14 to 16% Ca, and 4 to 6% Mg. If Cl and Na are determined, their percentage should each be less than 10%. Adjustments in available nutrient levels can be made by adding 75 g calcium nitrate (15-0-0) per cubic meter (2 oz $yd^{-3}$) to increase the test level by 10 ppm $NO_3$-N; 600 g (0-46-0) per cubic meter (1 lbs $yd^{-3}$) to increase the test level by 5 ppm P; and 55 g potassium nitrate per cubic meter (1.5 $yd^{-3}$) to increase the test level by 10 ppm K (Warncke, 1976 and 1979; Warncke and Krauskopf, 1983).

## Effects of Storage

1. Storage of prepared root media in either the dry or moist state will influence the soluble $NO_3$-N and soluble salt levels. If samples will not be extracted within 2 h of receipt, store them under refrigeration (~4$^{o}$C).

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*Chapter 7.2*

# Potential Acidity from Pyritic Sulfur in Soils

*F.J. Sikora and G. Huluka*

## Application and Principle

Reduced sulfur is prevalent in soils developed under anoxic conditions such as marsh environments and in geologic materials such as overburden remaining after surface coal mining, certain unweathered Coastal Plain sediments, organic rich sedimentary rocks and in some metamorphic strata. The reduced sulfur commonly exists as pyrite ($FeS_2$). Sulfur will remain in pyrite as long as it is not exposed to air. However, if sulfidic materials are drained or excavated, the sulfur in pyrite will oxidize to form sulfuric acid forming an acid sulfate soil. Sulfide bearing soils under marsh conditions, or materials that have been isolated at depth from the atmosphere, are commonly referred to as potential acid sulfate soils and typically have soil pH between 6.5 and 7.5 if they remain isolated from direct oxidation. After oxidation of sulfur in pyrite, soil pH may drop to less than 4 within a matter of weeks and the acidity may severely hamper plant growth and local water quality. Low soil pH causes problems when reclaiming land after surface coal mining or using potential acid sulfate soils for landscape development.

Lime can be applied to counteract soil acidity produced from sulfur oxidation. A typical analysis of soil for lime recommendation with a conventional soil pH or a soil/buffer pH method is not appropriate for sulfidic materials because these methods for agricultural soils do not accurately assess total potential acidity due to the presence of reduced sulfur. Two types of analyses can be utilized to assess potential acidity and then predict the lime requirement to neutralize the acidity. One method is referred to as acid-base accounting (Skousen et al., 2002). Total sulfur is analyzed in the soil and assumed to be all in pyritic-sulfur form. The maximum potential acidity is calculated from the stoichiometric reaction of sulfur with oxygen. Alkalinity is determined by reacting soil with a standard acid solution. The difference between initial acid concentration of the solution and the acid concentration of the solution after reaction with soil alkalinity is referred to as net soil alkalinity or neutralization potential. Soil alkalinity is referred to as net alkalinity because readily reactive soil acidity from aluminum may be present to neutralize total alkalinity. Net soil alkalinity can neutralize some or all of the maximum potential acidity produced from sulfur oxidation. Therefore, potential acidity in the acid-base accounting method is determined as the difference between maximum potential acidity and net soil alkalinity. The other method to assess potential acidity involves oxidizing soil sulfur with hydrogen peroxide and determining the net amount of acidity produced. A separate test for alkalinity is not required because any soil alkalinity is assumed to react with acidity produced from sulfur oxidation. The measured acidity is referred to as peroxide potential acidity. A review and evaluation of various methods for potential acidity are presented by Orndorff (2001).

The methods presented here are the acid-base accounting method and the peroxide potential acidity method. The acid-base accounting method is also documented by Sobek et al. (1978). Peroxide potential acidity is a method originally developed by Barnhisel and Harrison (1976) with examples of its use in Orndorff and Daniels (2004) and Orndorff et al. (2008).

# Potential Acidity from Acid-Base Accounting

## Maximum Potential Acidity from Total S

### Equipment and Apparatus

1. Jaw crusher grinder
2. Pulverizing grinder
3. Vario Macro Cube Elementar or other instrument suitable for combustion S analysis
4. Analytical balance sensitive to 0.0001 g

### Reagents

1. Tungsten oxide powder ($WO_3$)
2. Refer to instrument's operating manual for required oxidation and carrier gases and list of consumable chemicals required for oxidation and reductions tubes.

### Procedures

1. Air-dry the sample.
2. If the sample contains rocky material, mix sample well and place in a jaw crusher grinder to crush the sample into particles that are a quarter inch or less. The sample is then ground through a pulverizing grinder until the entire sample passes through a 10-mesh screen. No part of the sample is discarded.
3. The whole sample is riffled into two subsamples. One subsample is placed in the original container for storage and the other subsample is ground through a pulverizing grinder or micromill until the entire subsample passes through an 80-mesh screen. No part of the subsample is discarded.
4. Follow the manufacturer's operating instructions to calibrate the instrument.
5. Weigh approximately 0.2 g of sample into a tin foil. The exact weight to 0.0001 g is recorded by instrument software for determining S concentration.
6. Add tungsten oxide ($WO_3$) powder to the sample at 1:1 to 1:3 sample:$WO_3$ ratio and mix well.
7. With a Vario Macro Cube, the sample is combusted in pure oxygen at 1150$^{o}$C to produce sulfur dioxide ($SO_2$) which is purified before it reaches the detector. Complete combustion is enhanced by adding tungsten oxide ($WO_3$) to the sample. A thermal conductivity detector is used to quantify the $SO_2$ evolved from the sample.
8. Various combustion instruments are available for S analysis. Follow the operating procedures of the instrument's manufacturer for sulfur analysis.

### Calculations

1. Maximum potential acidity (MPA) is determined from total S.

   Maximum potential acidity (tons $CaCO_3$ (1000 tons material)$^{-1}$ ) = - (%S × 31.25)

The maximum potential acidity is expressed as a negative value because the unit represents alkalinity (tons $CaCO_3$ (1000 tons material)$^{-1}$ ). Acidity due to S is considered negative alkalinity. One thousand tons of material is equal to the amount of soil at a 6 inch depth in one acre with soil bulk density of 1.47 g cm$^{-3}$.

## Analytical Performance

Range and Sensitivity

1. Detection range is 0.02 to 15 % S.

Precision and Accuracy

1. Precision is less than 0.05 % S.

Interferences

1. Sodium and other alkali metals may interfere with complete combustion.

## Interpretation

1. The absolute value of maximum potential acidity (see Calculations, 1.) is the tons of $CaCO_3$ needed per 1000 tons of material to neutralize the acidity. For samples with no alkalinity, this would be the amount of $CaCO_3$ required to neutralize acidity. Consult the next two sections for determining potential acidity if the samples are suspected to contain alkalinity.

## Safety and disposal

1. The chemicals used in this procedure should be stored and disposed of according to routine laboratory procedures.

# Net Alkalinity

## Equipment and Apparatus

1. 250-mL Erlenmeyer flasks (2 required per sample and 2 required for blank)
2. 50-mL beaker (1 required per sample)
3. 100-mL burets (one for acid and one for base)
4. Hot plate (steam bath can be substituted)
5. pH meter
6. Analytical balance with 0.0001 g resolution
7. 1 L bottles equipped with ascarite columns (4 required)

## Reagents

1. *Potassium hydrogen phthalate ($KHC_8H_4O_4$)*
2. *$CO_2$-free water*: Boil water to expel $CO_2$ and let cool.
3. *Sodium hydroxide (NaOH), approximately 0.5 M*: Dissolve 20.00 g of NaOH pellets in $CO_2$-free water and dilute to 1 L with $CO_2$ free water. Protect with ascarite column. Standardize with potassium hydrogen phthalate.
4. *Sodium hydroxide (NaOH), approximately 0.1 M*: Dissolve 200 mL of 0.5 *M* NaOH to a volume of 1 L with $CO_2$ free water. Protect with ascarite column. Standardize with potassium hydrogen phthalate.
5. *Hydrochloric acid (HCl), approximately 0.5 M*: Dilute 42 mL of concentrated HCl to a volume of 1 L with $CO_2$-free water. Protect with ascarite columns. Standardize with a standardized solution of NaOH that is approximately 0.5 *M*.
6. *Hydrochloric acid (HCl), approximately 0.1 M*: Dilute 200 mL of the 0.5 *M* HCl to 1 L with $CO_2$-free water. Protect with ascarite columns. Standardize with a standardized solution of NaOH that is approximately 0.1 *M*.

## Procedures

1. Prepare sample as described in "Procedures" for "Maximum Potential Acidity from Total S".
2. Weigh approximately 2 g of 80-mesh sample into each of two 250-mL Erlenmeyer flasks. If sample pH in a 1:1 sample/water ratio is less than 1.0, reduce sample weight to 1 g. Record the exact weight to 0.001 g.
3. A preliminary effervescence test is carried out. Measure 4.5 $cm^3$ of dry 80-mesh sample into a 50-mL beaker, add 10 mL deionized water and stir with a glass rod to remove bubbles. Add 4 to 6 mL of 6 *M* HCl and stir with glass rod. Immediately observe surface for bubbling (effervescence). Effervescence is rated as 0, +, ++, +++, or ++++ indicating carbonates as none, few, numerous, very numerous, or extremely numerous.
4. Carefully add HCl in the amount and normality shown in Table 1 into one of the flasks. In the second flask, carefully dispense the amount and normality for the next higher rating (Table 1). Prepare two blanks with addition of HCl in the same amounts and normality to two Erlenmeyer flasks without sample.

*Table 1. Volume and molarity of HCl used, by effervescence rating.*

| Effervescence | HCl | |
|---|---|---|
| Rating | mL | Molarity |
| 0 | 20 | 0.1 |
| + | 40 | 0.1 |
| ++ | 60 | 0.5 |
| +++ | 80 | 0.5 |
| ++++ | 100 | 0.5 |

5. Heat the flask and hot plate to near boiling, swirling the flask at least every 5 min, until the reaction is complete.
6. The reaction is complete when gas evolution has stopped and the remaining sample particles will settle evenly over the bottom of the flask.
7. Add $CO_2$-free water to make a total volume in the sample flask of 125-mL.
8. Place sample on hot plate and boil for 1 min. Remove sample from hot plate and cool to 30$^{o}$C. Cover tightly and cool to room temperature. Do not place stopper in the hot flask as it may implode upon cooling.
9. Place pH electrode in flask and titrate with standardized NaOH with approximate concentrations of either 0.1 *N* or 0.5 *N*, whichever is closest to the normality of the HCl added before digestion in step 4. Titrate until a constant reading of 7.00 remains on the pH meter for at least 30 seconds.
10. If less than 3 mL of NaOH is required to obtain a pH of 7.00, it is likely the HCl added in the first flask was not sufficient. The duplicate sample should then be titrated and used for calculations.

## Calculations

1. Standardization of the NaOH requires the following calculation.

   NaOH molarity = (g $KHC_8H_4O_4$) ÷ (204.228 g mole$^{-1}$) ÷ (mL NaOH) × 1000 mL L$^{-1}$

2. Molarity of the HCl reacting with the soil is determined after standardization of the NaOH from the following.

   HCl molarity = (NaOH molarity) × (mL NaOH) ÷ (mL HCl)

3. Amount of acid consumed by the sample is determined from the following.

   mmole HCl consumed =
   (mL NaOH titrating blank – mL NaOH titrating sample) × NaOH molarity

4. Net alkalinity is determined as the calcium carbonate equivalence per 1000 tons of material.

   Tons $CaCO_3$ (1000 tons material)$^{-1}$ = (mmole HCl consumed) × 50.04 ÷ (sample wt, g)

   The alkalinity determined is net alkalinity (NA) because acidity not associated with sulfide oxidation may be present in the material and neutralize a portion of the alkalinity released. Sulfide minerals do not oxidize and produce acidity during the test. However, minerals such as jarosite ($KFe_3(SO_4)_2(OH)_6$) may produce acidity upon dissolution or acidity may be present as $H^+$ on the surface of minerals or organic matter. Siderite ($FeCO_3$) may produce erroneously high net alkalinity due to carbonate release during dissolution in HCl. In the field, siderite dissolution has a neutral affect on alkalinity production because ferrous iron oxidation and precipitation as ferric hydroxide generates acidity to neutralize alkalinity from released carbonate. Net alkalinity is positive if carbonate release exceeds acidity in the

material. Net alkalinity may be negative if acidity in the material exceeds carbonate minerals.

## Analytical Performance

Range and Sensitivity

1. Net alkalinity can range from 2.5 tons $CaCO_3$ (1000 tons material)$^{-1}$ with as little as 0.1 mmole HCl consumed to as high as 1250 tons $CaCO_3$ (1000 tons material)$^{-1}$ with 50 mmole HCl consumed.

# Potential Acidity from Total S and Alkalinity

## Calculations

1. The potential acidity is determined as the sum of maximum potential acidity as percent S and net alkalinity.

   Potential Acidity (tons $CaCO_3$ (1000 tons material)$^{-1}$) =
   maximum potential acidity + net alkalinity

   A summation is required because maximum potential acidity and net alkalinity have the same unit of tons $CaCO_3$ (1000 tons material)$^{-1}$. Maximum potential acidity will be negative with the value being the equivalent $CaCO_3$ required to neutralize the acidity. The magnitude of the negative value for maximum potential acidity is lessened in the value of potential acidity due to the presence of alkalinity in the material.

## Analytical Performance

Range and Sensitivity

1. Ranges were observed for potential acidity, maximum potential acidity, and net alkalinity from 14 diverse samples of sulfide-bearing material sampled from road cuts and excavations in Virginia as shown in Table 2.

*Table 2. Average and range of acidity and alkalinity values (tons $CaCO_3$ (1000 tons material)$^{-1}$) for 14 sulfide-bearing materials from road cuts and excavations in Virginia (Orndorff, 2001)*

| | Average | Minimum | Maximum |
|---|---|---|---|
| Potential acidity | -57.4 | -125 | -12 |
| Maximum potential acidity (MPA) | -58.7 | -128 | -16 |
| Net alkalinity (NA) | -1.3 | -15.2 | 16.6 |

## Interpretations

1. Potential acidity is a negative value in units of tons $CaCO_3$ (1000 tons material)$^{-1}$ and the absolute value represents the amount of lime needed to neutralize potential acidity. Even when alkalinity exceeds acidity, long-term experience in the Appalachian coalfields indicates certain materials that appear to be alkaline in terms of acid-base accounting may still produce acidic soil and water conditions due to quicker oxidation of pyrite compared to the slower rate of carbonate dissolution and the potential for carbonates in the system to become coated with Fe-oxides which renders them non-reactive. For this reason, Skousen et al. (2002) recommend the ratio of added alkalinity from lime to maximum potential acidity be greater than 1 with a ratio of 2 or higher to be completely confident of long-term liming efficacy.
2. Analysis of potential acidity using acid-base accounting follows some assumptions as shown below.
   a. All the sulfur in the total sulfur (S) analysis is pyritic S.
   b. All the pyritic S will oxidize to form acid.
   c. All the net alkalinity determined with lab analysis will generate the same quantity of net alkalinity in the field.
   d. The rate of acidity produced from pyritic S oxidation will be similar to the dissolution rate of alkaline minerals.
3. If a significant portion of the S occurs in sulfate or organic forms, potential acidity will be overestimated since the S is assumed to be present as sulfide with release of 2 moles of $H^+$ per mole of S oxidized. Organic S will not produce acidity. Some sulfate minerals will release $H^+$ upon dissolution but the amount of acidity released is not as great as occurs with sulfide oxidation. Jarosite is a sulfate mineral that will release 1.5 moles of $H^+$ per mole of sulfate-S dissolved. Reactions of sulfide oxidation from iron pyrite ($FeS_2$) and dissolution of jarosite ($KFe_3(SO_4)_2(OH)_6$) are shown below.

   $$4\ FeS_2\ (s) + 30\ H_2O + 15\ O_2 \rightarrow 2\ Fe_2O_3\ (s) + 8\ SO_4^{2-} + 22\ H_2O + 16\ H^+$$

   $$2\ KFe_3(SO_4)_2(OH)_6\ (s) \rightarrow 3\ Fe_2O_3\ (s) + 4\ SO_4^{2-} + 2\ K^+ + 3\ H_2O + 6\ H^+$$

4. The amount of $CaCO_3$ needed to neutralize acidity is the amount of pure calcium carbonate, or calcium carbonate equivalence. The amount of agricultural lime to apply is determined by knowing the effective neutralizing capability of the lime which is affected by chemical quality (percent calcium carbonate equivalence) and particle size (smaller particles have more complete dissolution). A common effective neutralizing capability of agricultural limestone is 67% which results in agricultural lime applications that are 1.5 times greater than the $CaCO_3$ application rate.
5. The amount of $CaCO_3$ needed to neutralize acidity is reported per ton of material. To convert the value to an area basis, such as acres, determine the bulk density of the material in the field and a soil depth needing neutralized. For a bulk density of 1.47 g cm$^{-3}$ and a soil depth of 6 inches, 1000 tons of soil is present in one acre.

# Peroxide Potential Acidity

## Equipment and Apparatus

1. Hot-water bath
2. Hot plate
3. 18-L bottle with ascarite column
4. 4.5-$cm^3$ scoop
5. 50-mL beaker
6. Glass stirring rod
7. 500-mL beaker
8. Ribbed cover glasses for 500-mL beakers
9. pH meter
10. Titration buret
11. Separating riffle

## Reagents

1. *Hydrogen peroxide, 30%*. Keep refrigerated.
2. *Potassium hydrogen phthalate ($KHC_8H_4O_4$).*
3. *Sodium hydroxide, 0.02 to 0.05 M*: Add 30 mL of a 1:1 (w/v) NaOH solution to 18 L of boiled, deionized water. Standardize with $KHC_8H_4O_4$ and store in stoppered bottle with ascarite column.
4. *Standard pH 4.0 buffer*
5. *Standard pH 7.0 buffer*

## Procedures

1. Prepare sample as described in "Procedures" for "Maximum Potential Acidity from Total S".
2. Weigh approximately 5 g of dry 80-mesh sample into a 500-mL beaker and cover with ribbed cover glass. Record the exact weight to 0.01 g.
3. Place beaker with sample into hot-water bath at 50°C.
4. Add 10 mL $H_2O_2$ to samples and wait until reaction stops. Continue to add $H_2O_2$ by 10 mL increments until 120 mL of $H_2O_2$ has been added. This procedure may require 3 to 4 h.
5. Keep reaction at 50°C for the rest of the day and night.
6. Remove beakers from water bath the next morning and place on hot plate at 95° C. Boil with ribbed cover glass in place until all effervescence has stopped. Add deionized water when volume drops to 50 mL. Do not allow samples to boil dry.
7. Remove samples from hot plate, cool to room temperature, and add deionized water until total volume is 150 mL. Use some of the deionized water to rinse adhered material from the sides of the beaker.
8. Titrate the sample with standardized NaOH to pH 7.0 using a standardized pH meter. Fill and empty the titration burette before titrating the sample.
9. A calculation should be made to determine if the procedure should be repeated with a smaller sample size:

a. mL NaOH × normality × 15 = tons of ag lime $acre^{-1}$
b. If the answer is greater than 20, then repeat steps 2 through 8 with duplicate samples using approximately 1 g sample. Record sample weight to 0.0001 g.

10. If the procedure using 1.00 g samples is followed, a second calculation should be used to determine if the sample size should be further reduced:
    a. mL NaOH × normality × 75 = tons of ag lime $acre^{-1}$
    b. If the answer is greater than 100, then repeat steps 2 through 8 with duplicate samples using approximately 0.2 g sample. Record sample weight to 0.0001 g.

## Calculations

1. Standardization of the NaOH requires the following calculation.

   NaOH molarity = (g $KHC_8H_4O_4$) ÷ (204.228 g $mole^{-1}$) ÷ (mL NaOH) × 1000 mL $L^{-1}$

2. Determine peroxide potential acidity with the following calculation.

   Peroxide potential acidity (Tons $CaCO_3$ (1000 tons material)$^{-1}$) =
   - (mL NaOH) × (molarity of NaOH) × (50) ÷ (sample size, g)

   Peroxide potential acidity is presented as a negative value since the unit represents alkalinity (tons $CaCO_3$ (1000 tons material)$^{-1}$). Any acidity measured is negative alkalinity. The absolute value is the tons of $CaCO_3$ needed per 1000 tons of material to neutralize the acidity.

## Analytical Performance

Range and Sensitivity

1. For 5 g sample, each 1 mL of NaOH translates to approximately 0.5 tons $CaCO_3$ (1000 tons material)$^{-1}$.
2. For 1 g sample, each 1 mL of NaOH translates to approximately 2.5 tons $CaCO_3$ (1000 tons material)$^{-1}$.
3. For 0.2 g sample, each 1 mL of NaOH translates to approximately 12.5 tons $CaCO_3$ (1000 tons material)$^{-1}$.

Precision and Accuracy

1. Peroxide potential acidity on a quality control sample used at the University of Kentucky soil test laboratory averaged 1.71 tons $CaCO_3$ (1000 tons material)$^{-1}$ with a standard deviation of ±0.30 for 8 measurements tested in analysis runs on different days.

## Interpretation

1. The absolute value of the peroxide potential acidity is the amount of pure $CaCO_3$ required to neutralize acidity from sulfide oxidation. Agricultural lime is the usual source of $CaCO_3$. The amount of agricultural lime recommended for application depends on the effective neutralizing capability of the lime. Agricultural lime commonly has 67% effective

neutralizing capability which results in the following calculation to determine the amount of agricultural lime to apply.

Peroxide potential acidity (Tons agricultural lime (1000 tons material)$^{-1}$) =
- (mL NaOH) × (molarity of NaOH) × (75) ÷ (sample size, g)

The correction factor changed from 50 for pure $CaCO_3$ to 75 for agricultural lime. For agricultural lime having other effective neutralizing capabilities, the correction factor can be determined as shown below:

Correction factor = 50 × 100 ÷ (percent effective neutralizing capability)

2. The bulk density may be greater from a surface disturbed by mining than from an agricultural soil. A common bulk density for agricultural soil in the surface 6 inches is 1.47 g $(cm^3)^{-1}$ which is equivalent to 1000 tons of soil in one acre. The bulk density of a reconstructed surface in land reclamation and depth of treatment can be evaluated to convert application rates from a mass basis to an area basis.
3. Any alkalinity from carbonate minerals in the material tested is assumed to dissolve and neutralize a portion of the acidity produced from sulfide oxidation. Therefore, a separate analysis of alkalinity of the material is not required as conducted in the acid-base accounting method.
4. The method assumes that all pyritic sulfide reacts with $H_2O_2$. This assumption may not be accurate. An analysis of standards with known pyrite contents indicated samples with sulfur concentrations greater than 0.9% resulted in 75 to 90% of the predicted value from complete oxidation of sulfide (Orndorff, 2001). Even with incomplete reaction with pyritic sulfide, the method was adequate to predict lime needs for successful revegetation of sulfidic spoil material (Orndorff et al., 2008).
5. Because of erratic results on pyritic overburden in Texas, O'Shay et al. (1990) modified an $H_2O_2$ oxidation procedure developed at West Virginia University (Grube et al., 1971; Sobek et al., 1978) to involved grinding material to a finer size (<149 µm), removing carbonate minerals with acid and 1 *M* $CaCl_2$ leaching prior to $H_2O_2$ oxidation, use of Cu to decompose excess $H_2O_2$, and extracting acidity with 1 *M* $CaCl_2$ after sulfide oxidation before titrating the filtrate with alkali.

## Effects of Storage

1. Air-dried soils may be stored several months without affecting measurements.
2. The electrodes used for pH measurement should be maintained and stored according to the manufacturer's instructions.

## Safety and disposal

1. The chemicals used in this procedure should be stored and disposed of according to routine laboratory procedures.

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