United States Environmental Protection Agency
Office of Water
Office of Environmental Information
Washington, DC
EPA-843-R10-002

National Wetland Condition Assessment

Laboratory Operations Manual

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1. INTRODUCTION

This manual describes laboratory protocols and is for use by laboratory personnel for samples associated with the National Wetland Condition Assessment (NWCA). The NWCA is one in a series of National Aquatic Resource Surveys (NARS) conducted by the Environmental Protection Agency (USEPA) to provide the public with a comprehensive assessment of the condition of the Nation's waters. In addition to wetlands, the NARS will assess coastal waters, lakes, rivers, and streams in a revolving sequence.

USEPA will collaborate with state, tribal, federal, and other partners to implement the NWCA to meet three goals:

1. Produce a report that describes the ecological condition of the Nation's wetlands.

2. Assist states and tribes in the implementation of wetland monitoring and assessment programs that will guide policy development and aid decision-making.

3. Advance the science of wetlands monitoring and assessment to support management needs.

The NWCA responds to the long-term goals outlined in USEPA's current strategic plan (USEPA 2006a) to improve the Nation's water quality (Goal 2.3) and to protect, sustain, and restore the health of critical natural habitats and ecosystems, including wetlands (Goal 4.3). Development of the NWCA will build on the accomplishments of the U.S. Fish and Wildlife Service (USFWS) and their production of national reports on status and trends in wetland acreage. When taken together, the NWCA and the USFWS *Wetland Status and Trends* (S&T) results will be used to measure progress toward attainment of the national goal to increase the quantity and quality of the Nation's wetlands. These complementary studies can influence how wetlands are managed at local, state, and national scales (Scozzafava, et. al. 2007).

The complete documentation of overall project management, design, methods, and standards for the NWCA is contained in four companion documents:

1. National Wetland Condition Assessment: Quality Assurance Project Plan (EPA-843-R10-003)
2. National Wetland Condition Assessment: Site Evaluation Guidelines (EPA-843-R10-004)
3. National Wetland Condition Assessment: Field Operations Manual (EPA-843-R10-001)
4. National Wetland Condition Assessment: Laboratory Operations Manual (EPA-843-R10-002)

This document (*Laboratory Operations Manual*) contains information on the methods for analyses of the samples collected during the project, quality assurance objectives, sample handling, and data reporting. Methods described in this document are to be used specifically in work relating to NWCA. All Project Cooperator laboratories should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. More details on specific methods for site evaluation, sampling, and sample processing can be found in the appropriate companion document.

The suggested citation for this document is:

USEPA. 2011. National Wetland Condition Assessment: Laboratory Operations Manual. EPA-843-R10-002. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.

1.1 Form Logistics

The National Wetland Condition Assessment will include laboratory processing of some indicators. Water chemistry, algae, vegetation, and soils samples will be sent to laboratories for analysis. These laboratories should fill out the applicable data forms (appendices) and email them to the Information Management Coordinator at NARSDataSubmission@epa.gov. If email is not available, forms may be shipped to:

Marlys Cappaert, SRA Intl.
c/o EPA
200 SW 35th Street
Covallis, OR 97331

1.2 Tracking Samples

With the samples from the field crews, laboratories should also receive tracking forms (refer to *NWCA Field Operations Manual* (FOM)). These forms will list the samples/specimens that should be included in the shipment. Laboratory personnel should cross check the forms with the samples received to verify that there are not any inconsistencies. If any sample/specimen is missing from the shipment, contact the Information Management Coordinator (541-754-4663) immediately.

The lab will adhere to strict sample tracking procedures to ensure samples are handled in an expeditious manner. Tracking procedures include, at a minimum, immediate notification of

shipment arrival followed within 24 hours by an electronic listing of the samples received. Results are reported in an electronic format detailed in the *MAIA-Estuaries Data Format Manual*. A copy of the cover letter which accompanies the results shall be sent to the EPA Contracting Officer.

1.3 Sending Resultant Data Forms

Resultant data files (i.e., EXCEL spreadsheets) are also emailed to the Information Management Coordinator at NARSDataSubmission@epa.gov for the purpose of quality assurance. A written report detailing the methods used discussing problems encountered must be included as an appendix to this report. Original records, such as laboratory notebooks, shall be retained for at least five years following final report submission, as they may be requested to be sent to EPA. If email is not available, forms the report may be shipped to:

Marlys Cappaert, SRA Intl.
c/o EPA
200 SW 35th Street
Covallis, OR 97331

1.4 References

USEPA. (1997) MAIA-Estuaries 1997 Laboratory Data Format and Transmittal Guidance Document. United States Environmental Protection Agency, Atlantic Ecology Division. Narragansett, RI.

Scozzafava, M.E., T.E., Dahl, C., Faulkner, and M. Price. 2007. Assessing status, trends, and condition of wetlands in the United States. National Wetlands Newsletter 29:24-28.

2. WATER CHEMISTRY

2.1 Introduction to Indicator

Along with vegetation and soil, water is one of the critical media for defining a wetland. Some studies show that water chemistry analyses are useful for evaluating wetland ecological integrity and evaluating stressor-response relationships (Lane and Brown, 2007; Reiss and Brown, 2005). Categories of data to be collected include the following:

1. pH
2. Nutrient Enrichment
3. Dissolved oxygen
4. Temperature

For more detailed information please see the report *Ecological Indicators for the 2011 National Wetland Condition Assessment* (in preparation).

2.2 Parameters for the NWCA

A total of four parameters will be measured from each water sample collected (Table 2.2-1). Table 1 also lists example methods that have been demonstrated to achieve the required objectives. Figure 1 provides a flow diagram of water chemistry processing procedures.

Table 2.2-1. Water chemistry parameters measured for the National Wetland Condition Assessment.

Analyte	Units	Example Method(s)[1]	Method Detection Limit Objective[2]
Conductivity	µS/cm at 25˚C	EPA 120.6 (conductivity cell)	NA
pH	pH units	EPA 150.6 (modified) (Aliquot from bulk water sample)	NA
Ammonia (NH_3)	mg N/L	EPA 350.1, or modifications (e.g., Automated Colorimetric, or modified to use salicylate and dichloroisocyanurate with analysis by flow injection analyzer (FIA))	0.02
Nitrate-Nitrite (NO_3^- NO_2^-)	mg N/L	EPA 353.2 (modified) (Automated colorimetric analysis with cadmium reduction by FIA)	0.02

Table 2.2-1. Water chemistry parameters measured for the National Wetland Condition Assessment.

Analyte	Units	Example Method(s)[1]	Method Detection Limit Objective[2]
Total Nitrogen (TN)	mg/L	EPA 353.2 (modified) (Persulfate Digestion; Automated colorimetric analysis with cadmium reduction; modified for analysis by FIA)	0.01
Total Phosphorous (TP)	µg P/L	EPA 365.1 (modified) Persulfate digestion	2

[1] Methods presented are those used for WSA analyses (and have met the performance characteristics listed). In some cases, a potential alternative method is also presented. Methods presented here should not be interpreted as the required method(s) to be used by all laboratories analyzing NWCA samples.

[2] The method detection limit is determined as a one-sided 99% confidence interval from repeated measurements of a low-level standard across several calibration curves.

2.3 Performance-based Methods

As an alternative to specifying laboratory methods for sample analysis, we use a performance-based approach that defines a set of laboratory method performance requirements for data quality. Method performance requirements for this project identify detection limit, precision, and accuracy objectives for each parameter (Table 2.3-1). It should be noted that there are no required methods to be used by all participating laboratories. Laboratories may choose to use other analytical methods for any target analytes as long as they are able to achieve the same performance requirements as listed in Table 2.3-1.

2.4 Sample Processing and Preservation

Upon receipt of samples, inspect each sample and complete the tracking form before storing in the refrigerator at 4°C until aliquots are ready to be prepared. Figure 1-1 illustrates the sample preparation procedures, including filtering and acidifying, for the various analytes.

Filter the aliquots for ammonia through 0.4µm pore size polycarbonate filters within 48 hours of arrival at the Laboratory. Rinse vacuum filter funnel units thoroughly with reverse-osmosis (RO) or de-ionized (DI) water five times before each use and in between samples. After placing a filter in the funnel unit, run approximately 100 ml of RO or DI water through the filter, with vacuum pressure, to rinse the filter. Discard the rinse water, then place the appropriate sample bottle under the funnel unit and filter the sample directly into the bottle. If a new filter is needed, remove the sample bottle, and rinse the new filter with 100 ml of RO or DI water before continuing sample filtration.

After all filtered and unfiltered aliquots are collected add ultra-pure acid (H_2SO_4) to the sample in the aliquot container. Store all aliquots in a refrigerator at 4°C for a holding time of 28 days.

Table 2.3-1: Performance requirements for water chemistry analytical methods

Analyte	Units	Potential Range of Samples[1]	Long-Term MDL Objective[2]	Laboratory Reporting Limit[3]	Transition Value[4]	Precision Objective[5]	Bias Objective[6]
Conductivity	µS/cm at 25˚C	1 to 15,000	NA	2.0	20	± 2 or ±10%	± 2 or 5%
pH	pH units	3.7 to 10	NA	NA	5.75 and>8.25	± 0.08 or ± 0.15	± 0.05 or ± 0.10
Ammonia (NH_3)	mg N/L	0 to 17	0.01 (0.7 µeq/L)	0.02 (1.4 µeq/L)	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	0 to 360 (as nitrate)	0.01	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Nitrogen (TN)	mg/L	0.1 to 90	0.01	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Phosphorus (TP)	µg P/L	0 to 22,000	2	4	20	± 2 or ±10%	± 2 or ±10%

1 Estimated from samples analyzed at the WED-Corvallis laboratory between 1999 and 2005 for TIME, EMAP-West, and WSA streams from across the U.S.

2 The long-term method detection limit is determined as a one-sided 99% confidence interval from repeated measurements of a low-level standard across several calibration curves, based on USGS Open File Report 99-193. These represent values that should be achievable by multiple labs analyzing samples over extended periods with comparable (but not necessarily identical) methods.

3 The minimum reporting limit is the lowest value that needs to be quantified (as opposed to just detected), and represents the value of the lowest nonzero calibration standard used. It is set to 2x the long-term detection limit, following USGS Open File Report 99-193 New Reporting Procedures Based on Long-Term Method Detection Levels and Some Considerations for Interpretations of Water-Quality Data Provided by the U.S. Geological Survey National Water Quality Laboratory.

4 Value at which performance objectives for precision and bias switch from absolute (≤ transition value) to relative 9> transition value). Two-tiered approach based on Hunt, D.T.E. and A.L. Wilson. 1986. The Chemical Analysis of Water: General Principles and Techniques. 2nd ed. Royal Society of Chemistry, London, England.

5 For duplicate samples, precision is estimated as the pooled standard deviation (calculated as the root-mean square) of all samples at the lower concentration range, and as the pooled percent relative standard deviation of all samples at the higher concentration range. For standard samples, precision is estimated as the standard deviation of repeated measurements across batches at the lower concentration range, and as percent relative standard deviation of repeated measurements across batches at the higher concentration range.

6 Bias (systematic error) is estimated as the difference between the mean measured value and the target value of a performance evaluation and/or internal reference samples at the lower concentration range measured across sample batches, and as the percent difference at the higher concentration range.

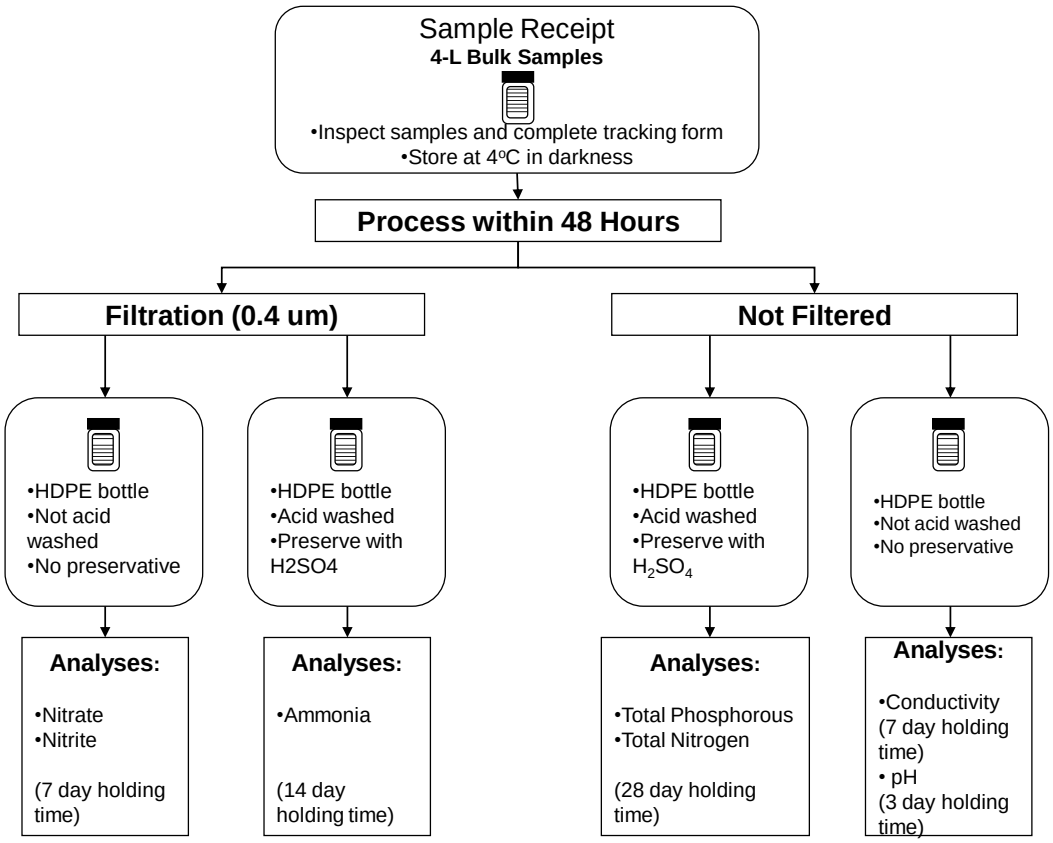

Figure 1-1. Water chemistry sample processing procedures.

2.5 Quality Assurance/Quality Control (QA/QC) Procedures

Since multiple laboratories will be analyzing water chemistry samples, specific quality control procedures must be implemented to ensure that 1) the data quality objectives are being met and 2) data is consistent and comparable among all participating labs. Specific QA/QC procedures will depend on whether or not the lab has been certified by the National Environmental Laboratory Accreditation Conference (NELAC). More stringent QA/QC requirements will be implemented for labs that are not NELAC certified.

A total of 10% of the samples collected will be re-analyzed for quality control. Re-analysis will be performed by another experienced technician at an independent laboratory who did not participate in the original analyses. EPA estimates that approximately 100 samples may require re-analysis for all analytes. EPA will inform the laboratories which random samples will be re-analyzed. The QC laboratory technician should complete another copy of the Water

Chemistry Datasheet for each sample. Each sheet should be labeled with the term "QC Re-analysis." As each sheet is completed, it should be emailed to the Information Management Coordinator at NARSDataSubmission@epa.gov.

EPA will compare the results generated by the primary and QC laboratories for each sample and calculate percent similarity (refer to Chapter 2 of the QAPP). It is expected that the two analyses should have a similarity of ≥90%. If not, the reasons for the discrepancies between laboratories should be discussed. Results less than these values will be investigated and logged for indication of error patterns or trends.

Significant differences may result in the re-analysis of samples by the primary laboratory and a second QC check by the secondary laboratory. All samples must be stored at the laboratory until the Information Management Coordinator notifies the lab.

2.5.1 Internal QA/QC Procedures

Prior to sample analysis, all laboratories will be required to provide internal QA documentation (e.g., Quality Management Plan, Quality Assurance Project Plan) for external review. QA documentation will be reviewed by the QA Coordinator to ensure consistency among all participating labs.

2.6 References

Hunt, D.T.E. and A.L. Wilson. 1986. The Chemical Analysis of Water: General Principles and Techniques. 2nd ed.. Royal Society of Chemistry, London, England.

Lane, C.R. and M.T. Brown. 2007. Diatoms as indicators of isolated herbaceous wetland condition in Florida, USA. Ecological Indicators 7(3):521-540.

Reiss, K.C. and M.T. Brown. 2005. The Florida Wetland Condition Index (FWCI): developing biological indicators for isolated depressional forested wetlands. A Report to the Florida Department of Environmental Protection. Howard T. Odum Center for Wetlands, University of Florida, Gainesville, Florida, USA.

Youden, W.J. 1969. Ranking laboratories by round-robin tests. In Precision Measurement and Calibration. H.H. Ku, ed. NBS Special Publication 300, Vol. 1. U.S. GPO Washington, D.C.

3. ALGAE

3.1 Introduction to Indicator

Algae, which include planktonic (open water), benthic (periphyton), and metaphyton forms, are an extremely important ecosystem component providing essential primary productivity as well as a resource for higher level organisms including macroinvertebrates and fish (Mitsch and Gosselink, 2007). Like other biotic taxa, many indicator attributes or metrics can be derived from data describing taxonomic composition and abundance of algae. Categories of data to be collected include the following:

1. Species composition and abundance
2. Guilds
3. Productivity
4. Habitat evaluation
5. Physiology

For more detailed information please see the report *Ecological Indicators for the 2011 National Wetland Condition Assessment* (in preparation).

Algae samples will be collected by field crews and sent to a laboratory for analysis on a regular basis during the field season to avoid delays in processing and identifying samples (for more information on shipping field samples, refer to FOM section 12.3). Laboratories should receive a 250ml composite sample of algae from benthic and vegetative surfaces that includes both diatoms and soft algae. This sample will be subsampled so the diatoms can be acid cleaned and analyzed separately from the subsample of composite algae that includes soft algae along with the diatoms (see Figure 3.1-1 for a flowchart of processing activities). Crews will also send a phytoplankton chlorophyll *a* sample from those assessment areas where standing water is present. This sample will have been filtered using a 0.4µm pore size glass filter in the field. Laboratories should receive the filter in a small vial that has been frozen. The following method for the analysis of composite algae samples has been adapted from *Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment program* (Charles et al., 2002) and is used to identify and enumerate algal populations. EPA recommends these procedures for the analysis of NWCA composite algae samples. However, we recognize that in some cases there are alternate methods that may produce comparable results. Laboratories interested in using alternate methods must submit them to EPA for approval prior to conducting any analyses (see QAPP for lab and personnel qualifications).

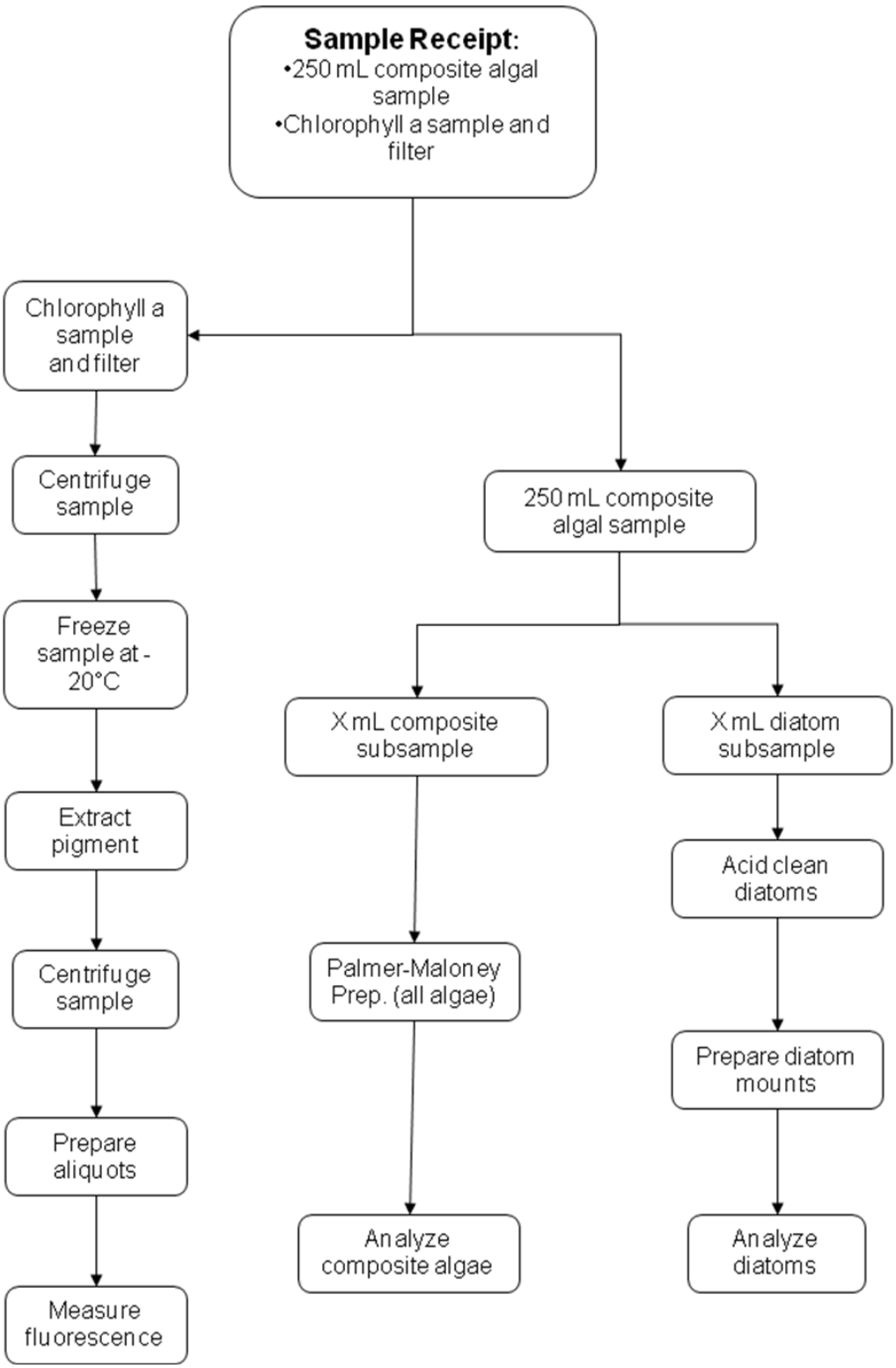

Figure 3.1-1: Soft algae, diatom, and Chlorophyll-*a* processing procedure.

3.1.1 References

Charles, D. F., C. Knowles, and R. Davis. 2002. Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. Patrick Center for Environmental Research Report No. 02–06. Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, Pennsylvania.

Mitsch, W.J., and J.G.Gosselink. 2007. Wetlands. 4th ed. New York:Wiley.

Stevenson, Jan. Personal communication August 11, 2009.

USEPA. 2008. National Rivers and Streams Assessment: Laboratory Methods Manual. EPA 841-B-07-010. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.

USEPA. 2006. Survey of the Nation's Lakes. Laboratory Methods Manual. EPA841-B-06-005. U.S. Environmental Protection Agency, Washington, DC.

3.2 Subsampling

Qualitative composite algal samples must be subsampled carefully to ensure that all algal forms are represented in the subsamples to be analyzed. The sample received from field crews may have large amounts of sand, silt, or other heavy material that can interfere with algal analysis. Therefore, this is a special procedure involving the subsampling of these samples. In this procedure, the liquid portion is subsampled by volume and the heavier material that is difficult to suspend is separated by mass. Protocols for subsampling samples with heavy sediments can be found in the NAQWA protocols (section 7.5, pages 20-21).

3.2.1 Safety Precautions

Samples will arrive at the lab in a Lugol's preservative (see Algae Chapter of FOM). Samples preserved with the appropriate amount of Lugol's solution will look like weak tea (Figure 3.2-1). If a sample does not contain a preservative call the Information Management Coordinator (541-754-4663) before handling. Upon receipt at the lab, add a few (1-3) drops of 100% buffered formalin to the samples to ensure preservation.

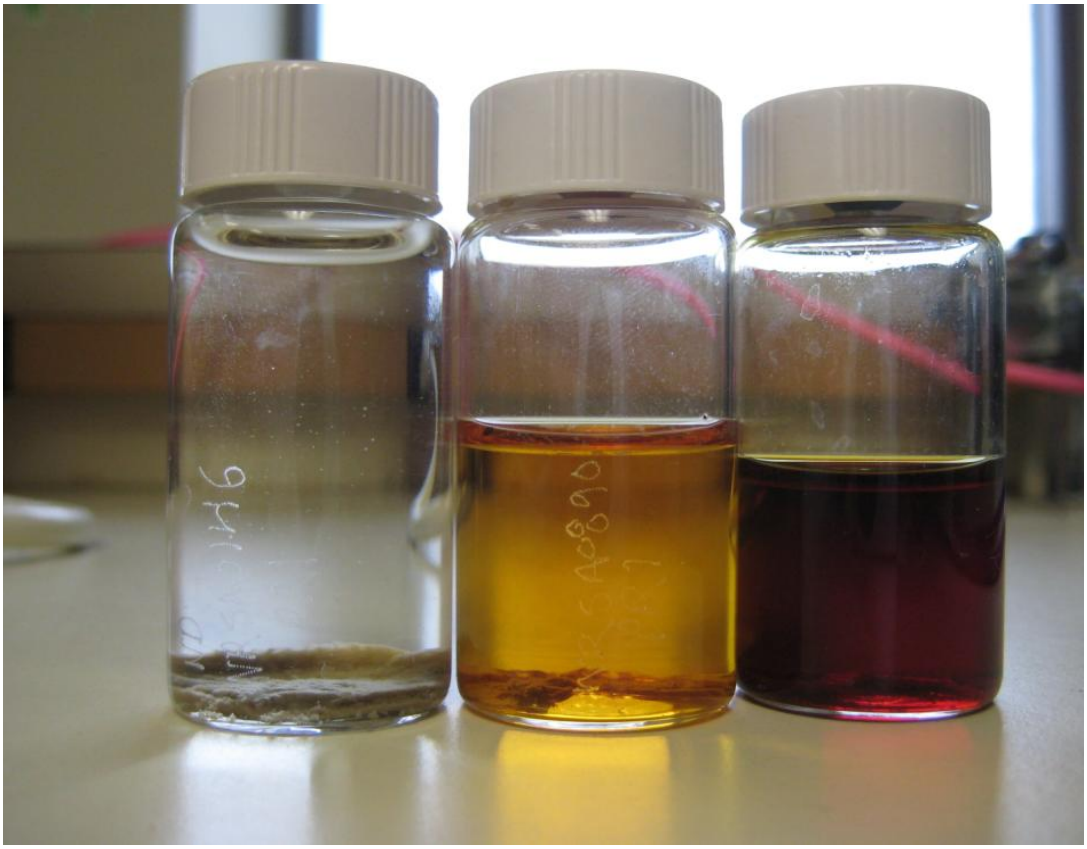

Figure 3.2-1: Image of algal samples preserved with Lugol's. The middle vial contains the appropriate amount of Lugol's while the left contains too little and the right contains too much. (Photograph from LaLiberte, 2010)

3.2.2 Recommended Apparatus/Equipment

- Distilled (DW) or reverse osmosis (RO) water
- Dispenser bottle for DW or RO water
- Beakers
- Beaker holding box (24 slots)
- Graduated cylinders (10 ml, 25 ml, 100 ml, 250 ml, 500 ml and 1 L)
- 20-ml vials
- 20-ml vial with 1 ml intervals marked on it for measuring volumes
- Diamond scriber
- Protective clothing (gloves, lab coat or apron, eye protection)
- Positive-draw fume hood
- Spatulas
- Plastic disposable pipettes (tips of plastic pipettes can be cut)
- Small turkey baster

3.2.3 Methods

1. The 250ml composite sample received from field crews must be separated into 2 subsamples for separate counting of diatoms and of all algae. Deciding how much to subsample for all algae and diatoms is somewhat of a judgment call. If the sample is very silty, it will be better to subsample a minimum of 10 ml for all algae, whereas if the sample looks clear, at least 20 ml is recommended. Sample at least 10 ml for soft and at least 20 ml for diatoms.

2. If there are visible macroalgal forms, these should have been cut into smaller pieces by the field crews and included in proper proportions in aliquots. If macroalgae were not cut by field crews, then they should be cut before subsampling.

3. Suspend the algal material by shaking or swirling and carefully pour the determined amount of subsample into a graduated cylinder. Ensure the sample stays well mixed during subsampling. Alternatively, use a wide-bore pipette or turkey baster to subsample the 125ml composite sample, which allows pumping the pipette or baster to mix the sample, while withdrawing an aliquot. Select a method that allows a representative subsample. Filaments and debris will block pipettes with small openings. Careful pouring or turkey basters may be more appropriate. Withdrawing multiple aliquots for each sample, each approximately 5 ml, enables drawing 2-4 aliquots for each 10-20 ml diatom and all-algae subsample.

4. Transfer each subsample to the 20-ml vial labeled with Subsample ID#.

5. Record the subsample volume and beaker ID of each subsample on Subsample Data Sheet.

3.2.4 Quality Assurance/Quality Control

Since algae are microscopic, there is significant possibility of contamination of samples. Laboratory rooms where raw samples are subsampled should be kept as clean as possible. Lab bench surfaces should be kept clean and free of debris.

The appropriate size of graduated cylinder for measuring samples and subsamples is critical. The sample should be at least one-third the capacity of graduated cylinder and the units of the

graduated cylinder should allow estimation to the nearest milliliter (finer for the small graduated cylinders used to measure a portion of a subsample).

3.2.5 References

Moulton, S.R., II, J.G. Kennen, R.M. Goldstein, J.A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities in the National Water-Quality Assessment program, U.S. Geological Survey Open-File Report 02-150. In Press.

Porter, S.D., T.F. Cuffney, M.E. Gurtz, M.R. Meador. 1993. Methods for collecting algal samples as part of the National Water-Quality Assessment program. U.S. Geological Survey Open-File Report 93-409, Raleigh, NC [39 pp] http://water.usgs.gov/nawqa/protocols/OFR-93-409/alg1.html

United States Geological Survey, National Water-Quality Assessment Program. 1997. Procedures for Processing NAWQA Algal Samples. Draft Manuscript. February 1997.

3.3 Diatom Cleaning

This protocol will apply to the subsample which will be analyzed for diatoms only. To identify and enumerate diatoms accurately at the species and variety levels, it is necessary to remove both extracellular and intracellular organic matter from the siliceous frustules of diatoms and other material such as the soft algae in the sample. Removing the organic matter is necessary so that all details of diatom structures essential to taxonomic identification are clearly visible. This protocol describes a method for removing organic material from a sample by digesting it with nitric acid.

3.3.1 Definitions

Digestion in this procedure refers to the solubilization of organic material by strong acid oxidation.

Diatom cells, called **frustules**, are composed of two **valves**. They have a siliceous structure, the features of which are used for taxonomic identification.

3.3.2 Safety Precautions

Nitric acid is an extremely hazardous reagent. As a strong acid oxidizer it can cause severe burning of exposed skin and clothing. At room temperature, concentrated nitric acid produces intense fumes when exposed to open air. Any concentrated nitric acid containers open to the air must be contained within a positive-draw fume hood at all times. There are no exceptions to this rule. Personnel are required to wear safety glasses, protective gloves and lab coats at all times when handling concentrated nitric acid. This is especially important when handling/venting the digestion vessels.

NWCA Samples delivered to the Diatom Preparation Laboratory will have been prepared with Lugol's solution (see section 3.2.2 of LOM Algae Chapter of FOM). This information should be on the shipping forms included with the samples or affixed to the shipping container.

Hot plate temperatures required for this procedure are high enough to cause severe burning of exposed skin. Use extreme care when manipulating slides on the hot plate and when working close to the hot plate.

3.3.3 Recommended Apparatus/Equipment

- Hot plate
- Positive-draw fume hood
- Safety glasses
- Acid-impervious hand protection
- Laboratory coat or apron, acid resistant
- DI water
- Siphon

3.3.4 Methods

1. The diatom subsample is digested under the hood on a hot plate.
    a. As an alternative the subsample can be digested in test tubes and heated in a sand bath.
    b. As another alternative the ANSP microwave digestion method can also be used (protocol P-13-42).

2. Set the hot plate on 250°C.

3. Cautiously add at least 50 ml nitric acid (under the hood) to the sample (rule minimum: 1:2 sample to acid ratio).
    a. Hydrogen Peroxide may be used as a less toxic alternative to nitric acid.

4. Heat the acid and sample mixture. Boil for 1 to 2 hours. Add DI water as needed to maintain the liquid levels.

5. Move the sample from the hot plate to a tray and let it cool for a few hours.

6. Very carefully add DI water (under the hood) up to the top of the beaker. Leave this overnight in the hood (for at least 8 hours).

    a. As an alternative, centrifuge samples for 10 minutes at approximately 2000 rpm.

7. Transfer the tray to the “water changing station”, siphon the water from the center of the water column (from side to side and top to bottom) to around 100 ml (the mark to which you remove water at this point can be variable). Avoid siphoning diatoms settled on the bottom or adsorbed to the sides of the beaker and water surface.

Note: Do not disturb the settled layer of sample on the bottom so none of the sample is lost. Also, diatoms adsorb to sides of the beaker and water surface at the top, so after siphoning add DI water, washing the sides of the beaker with a DI water bottle. Sprinkle water on the surface to dislodge algae adsorbed to the water surface. Leave the sample for at least 8 hours to allow time for the diatoms to settle in the beaker before washing again (the rule is 1 hour per 1 centimeter so the smallest diatoms can settle).

8. After a neutral pH is achieved (approximately 6-8 washings if samples are settled, 4-5 if samples are centrifuged), the digested diatom material is transferred to the pre-labeled vial for cleaned diatom material.

Note: The volume in the “diatom volume after digestion” vial is a judgment call by the laboratory technician. The goal is to achieve an even distribution for the slides in the next step. Tip: when shaken, the vial needs to be transparently cloudy. Leave some space for dilution in that vial. If the sample looks completely clear after digestion assume it is a sparse sample, and try to very carefully concentrate the whole sample to about 5 ml. It is easier to play with the volume than to count samples that are too sparse.

3.3.5 Quality Assurance/ Quality Control

Diatom frustules are microscopic, generally falling in the fine silt size range; therefore, there is a possibility that samples can be contaminated. Laboratory rooms where raw or processed samples are handled should be kept as clean as possible. Lab bench surfaces should be kept clean and free of debris. Techniques similar to those used for sterile experiments (bacteriological plating, etc.) should be followed to minimize the risk of cross contamination of samples. Where feasible, disposable pipettes, stirrers, etc. should be used. Where they cannot, they should be rinsed in DW or RO water at least three times after each sample. (Explanatory note: at times, tap water, because of algal blooms and use of diatomaceous earth filters, may contain diatoms.) All equipment should be stored dry to prevent growth of algae or fungi.

3.3.6 References

Acker, F., B. Russell, and E. Hagan. 2002. Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program: P-13-42 Diatom Cleaning by Nitric Acid Digestion with a Microwave Apparatus. The Academy of Natural Sciences. Report No. 02-06.

American Public Health Association, American Water Works Association, Water Environment Federation (APHA, AWWA, WEF). 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed.

Jan Stevenson, personal communication Aug. 11, 2009.

3.4 Preparation of Diatom Slides using Naphrax™ Mounting Medium

Accurate identification and enumeration of diatoms requires mounting of cleaned material between a microscope slide and coverslip in a medium with a high refractive index so that the features of diatom frustules or valves are clearly visible at high magnification. Naphrax™ is a commercially-available toluene-based mounting medium with high refractive index. This protocol details the steps necessary to produce high-quality diatom mounts from cleaned diatom material.

3.4.1 Safety Precautions

Naphrax™ should be considered a hazardous substance because it contains toluene, an organic solvent. Toluene volatilizes readily when heated. For this reason, the heating of Naphrax™ should only be performed under a positive-draw fume hood. Personnel should wear safety glasses and protective hand wear when working with liquid Naphrax™ at room temperature, when heating Naphrax™ in a hood, or when in contact with solidified Naphrax™ toward the final stages of slide preparation.

Hot plate temperatures required for this procedure are high enough to cause severe burning of exposed skin. Use extreme care when manipulating slides on the hot plate and when working close to the hot plate.

3.4.2 Recommended Apparatus/ Equipment

- Corning ceramic-top hot plate with temperature control
- Positive-draw chemical hood
- Aluminum drying plate (25.5 x 20.0 x 0.5 cm, solid aluminum; lines forming 48 squares, each 3.2 cm on a side, are etched on the surface. Each square is etched with an identifying number)
- Glass microscope slides (1 x 3 inches; 2.5 x 7.5 cm)
- Glass coverslips (18 mm x 18 mm or 22 mm x 22 mm) - No. 1 thickness, stored in covered glass jar filled with 100 % ethanol.
- Naphrax™ mounting medium
- Diamond scribe
- Disposable plastic pipettes
- Adjustable pipettor (0 - 250µl); adjustable pipettor (200 - 1000µl)
- Pipette tips for adjustable pipettors
- Round-style tooth picks
- Forceps
- Polished, rounded wooden splints
- Wash bottle filled with distilled (DW) or reverse osmosis (RO) water
- Single-edged razor blades
- Ethanol, 70%
- Acetone
- Kimwipe® tissues
- Slide labels
- Wax (the kind commonly used for candle making and canning foods)
- 10% HCl

3.4.3 Methods

Estimate the amount of cleaned diatom material to deposit on coverslip.

1. Starting with cleaned material contained within 20-ml glass vials, estimate the volume of suspended material that will need to be deposited ("dripped") on a coverslip to produce a slide of the appropriate cell density. The ideal density to be achieved on the final mount is somewhat subjective and is based on the amount of debris in the sample, the preferences of the slide analyst, and the way in which the slide is to be used (e.g., counting, documentation). Generally, between 10 and 20 diatom specimens should be present in a single high power microscope field (1000X). The number of specimens per field will need to be reduced if samples contain considerable amounts of silt. This estimate is referred to as the "drip count" (the amount of cleaned material to be placed on a coverslip). Accuracy of estimates improves with experience. In many cases, analysts will request that both a "heavy" slide (~40 cells/field at 400 – 450x magnification) and a "light" slide (~30 cells/field at 400 – 450x magnification) be made. When slidemaking is complete, record the final amounts dripped on the Diatom Enumeration Data Sheet. Also note observations of interfering materials (sand, silt, etc.) on this form.

2. In some cases, the number of diatoms in a sample is very sparse. This is usually because diatoms were rare in the habitats sampled or the sample bottles contain a small amount of material. In these cases, additional procedures (steps 3 to 5 below) are required to either make a satisfactory slide for analysis or to determine that analysis of a sample is not practical.

    a. If a satisfactory slide could be made by increasing the concentration of cleaned diatom material by two to five times, then do this by using a micropipettor to remove the required amount of water from the vial of material after it has been allowed to settle for at least eight hours. Spin the vial carefully while still resting on the table top early, after about 4 h of the 8 hour period, to dissociate diatoms that have adsorbed onto the side of the vial so they will settle. Record the concentration factor on the Diatom Enumeration Data Sheet.

    b. If a concentration of cleaned material greater than two to five times is required, then re-subsample the original sample (Section 3.2). Take a subsample of a size sufficient to prepare satisfactory slides. Use the entire remaining sample only if absolutely necessary. Digest the subsample and prepare a new vial of cleaned material (Section 3.3). Repeat step 1, above. If the concentration of cleaned material is still not sufficient, concentrate it, as described above. If still too dilute, combine the two

vials of cleaned subsample materials. Record steps, volumes, and final concentration factor on the Diatom Enumeration Data Sheet.

c. If, after following the steps above to concentrate the cleaned material, the density of diatoms on a coverslip still does not meet the criteria of 30 to 40 cells per field at 400 - 450x magnification, proceed to make the densest slide possible and take it to a diatom analyst to evaluate. The analyst will make a determination of whether it is practical to analyze the sample. He/she will quickly scan the slide in its entirety under 100x magnification, and estimate the total number of individuals on the slide. Then he/she will make his/her determination of whether the slide is countable, taking into account the density of diatoms, evidence of dissolution, and amount of debris (silt, clay, broken remains of diatoms and other siliceous organisms) that would make it difficult to identify specimens accurately. As a general guideline, if accurate identifications are possible, and at least 100 specimens could be counted within four hours, he/she should determine that the slide be analyzed; otherwise it should not. If the diatom analyst determines that the slide should not be counted, inform the Logistic Coordinator at 231-941-2230 immediately. Only under very special circumstances will an analyst be asked to take the extraordinary measure of counting a slide for a very long time (more than four hours). Record results of the diatom analyst's determination and rationale on the Diatom Enumeration Data Sheet.

d. When doing an evaluation of a slide with few diatoms, as described in the step above, a diatom analyst may occasionally see evidence suggesting that a sample contains lightly silicified diatoms that may not have survived the digestion process. In these rare instances, he/she may suggest that a "burn mount" be made to determine whether diatoms did exist in the original sample. (This is one reason why a small portion of the initial sample should always be saved, even for phytoplankton.) The burn mount procedure was used extensively to create slides for diatom analysis before the introduction of methods incorporating acids for the digestion of organic material. Even though this method does not rid sample material entirely of organic debris, diatoms on the slide can at least be identified as diatoms. For this method, follow the EPA (1973) procedure. Briefly, a known portion of the untreated sample is dripped onto a coverslip and allowed to dry at room temperature. When the sample is dry, it is placed onto a hot plate and left for about 30 min at approximately 570°C. The coverslip is mounted according to step 7, below. After it is prepared, have the burn mount slide examined by a diatom analyst. He/she will determine if diatoms are present and whether analysis of the slide is warranted. Slides prepared using the burn mount method cannot be counted if too much organic material remains on the slide. This is because it is not possible to make accurate taxonomic identifications. Generally, burn mounts are used only as a last resort, and to confirm that weakly

silicified diatoms are not present in the sample. Record information on all burn mount attempts, successful or unsuccessful, on the Diatom Enumeration Data Sheet. Include date, name of preparer, volume of subsample used, and whether diatoms were observed.

Deposit cleaned material on coverslip.

3. Use forceps to remove single coverslips from the storage container and carefully clean each by wiping with a Kimwipe®. Place each coverslip on a marked space. Be sure the drying area is clean and dry to avoid cross-contamination. If the intended drip count will be less than 600µl, drip an amount of distilled water onto the coverslip with a disposable pipette that will be sufficient to form a thin layer of water over the entire coverslip when the diatom suspension is added. Agitate the sample vial to homogenize the diatom suspension. Using an adjustable pipettor, quickly withdraw the required amount from near the central portion of the sample. Eject this material smoothly and carefully onto the layer of distilled water already on the slip. By alternately drawing material up into the pipette and ejecting it, a homogeneous suspension is achieved on the coverslip. In the case where more than ~600µl of original sample is required, the addition of distilled water is not necessary, and the sample can be ejected and mixed directly on the coverslip. In both cases, take care to ensure that the suspension covers the entire surface of the coverslip, including the extreme edges of the corners. Add 10% HCl to the cleared slurry (2 drops per 20ml of material in vial) to achieve a more even distribution on the cover slip.

4. Should the coverslip overflow, discard the coverslip, wipe the drying area, and repeat the procedure with a freshly cleaned coverslip. Discard the pipette tip when finished with each sample. Once the marked space is loaded with coverslip preparations, it should remain undisturbed until the coverslips are dry. At this point, drying of the slips can proceed at room temperature (a period of several hours will be required), or gentle heat (warm to the touch only) may be applied to hasten evaporation (a crook-neck lamp with incandescent light bulb placed 15 - 30 cm over the coverslips is one option or a slide maker provides heat). However, rapid evaporation with heating can produce strong patterns of diatoms settling on coverglasses, which should be avoided. Once completely dry, put the coverslips on the hot plate that has been preheated to 120 to 150ºF. Leave for three to five minutes. This procedure ensures that nearly all water is driven from the material on the coverslips and helps assure that the diatom frustules will adhere to the surface of the glass. Remove the coverslips from the hotplate and inspect them. If the pattern of diatoms distributed on any of the coverslips is not even and smooth, they should be re-dripped. If coverslip distributions seem unsatisfactory after repeated attempts, consult an algal analyst.

Mount coverslip on microscope slide.

5. Using a diamond scribe, etch microscope slides with Sample ID, Subsample ID and Slide Replicate ID.

6. Mount coverslip on slide using the following steps.

THE FOLLOWING STEPS MUST BE PERFORMED IN A POSITIVE-DRAW FUME HOOD!

7. Using a rounded wooden splint or disposable pipette, transfer a small amount of Naphrax™ (volume equivalent to two to four drops of water) to the central portion of the etched side of the microscope slide. Then remove the appropriate coverslip from the aluminum plate with forceps, being careful to handle the coverslip only at the extreme corners. Invert the slip and place it gently on the Naphrax™ covered portion of the slide. Then place the slide (coverslip up) on the hotplate and apply gentle heat until the evolution of bubbles resulting from the evaporation of the toluene solvent first occurs, and then significantly diminishes. Heating the slide will allow the Naphrax to flow under the coverslip. Remove the slide from the hot plate, and, using the rounded toothpicks, gently position the coverslip and press it to form a uniform, thin layer of Naphrax™ beneath the entire coverslip. Make sure that the edges of the coverslip are brought parallel to the edges of the microscope slide. Care must be taken at this stage not to press so hard as to damage or dislodge the diatoms or cause warping of the coverslip. As this procedure is taking place, the Naphrax™ is “setting up” (becoming hard), and the ability to move the coverslip will diminish rapidly. At this point, set aside the mount to finish cooling.

8. Use a single-edge razor blade to carefully trim any excess Naphrax™ which has been squeezed out from beneath the coverslip. Great care must be taken to avoid “lifting” the coverslip by inadvertently allowing the edge of the blade to move between the coverslip and the microscope slide. Once most of the excess Naphrax™ has been removed and discarded, and while still working under the hood, place the mount in successive baths of acetone, and then ethanol for no more than 10 or 15 seconds each. Finally, wipe the mount clean with a Kimwipe® tissue.

9. Add a paper label to slides before slides have been analyzed.

10. Assemble forms and transmit slides. Put slides in plastic slide boxes; label each with name of project and subproject, Subproject ID, box _ of _, date (month/year) box prepared, and name or initials of preparer.

11. Preserve and store cleaned material. After slides are analyzed according to the appropriate protocol, and no additional slides need to be made, process the vials containing the remaining acid-cleaned material for long-term storage. Working under a fume hood, add two to four drops of 100% buffered formalin to each vial (some contractors use alcohol as a preservative instead). Adding glass beads to the vials will also help to preserve the diatoms. Tightly cap the vials and seal them by immersing the top 1/3 of the vial in melted wax. Then transfer the vials to the appropriate storage cabinet for long-term storage. Be sure that the cabinet and shelves on which they are stored are properly labeled with the study name, year, and Subproject ID.

3.4.4 Quality Assurance/ Quality Control

It should be understood that, given the microscopic size and large numbers of diatoms which are transferred from the cleaned material vials to the finished mount, there are a number of steps where contamination of the samples is possible. Laboratory rooms where raw or processed samples are handled should be kept as clean as possible. Laboratory bench surfaces should be kept clean and free of debris at all times. Techniques similar to those used for sterile experiments (bacteriological plating, etc.) should be followed to minimize the risk of cross-contamination of samples. All equipment coming into contact with sample material should be rinsed in DW or RO water at least three times. Disposable pipettes should be used when possible.

The distribution of specimens on the final mounted coverslips should represent the samples contained within the cleaned material vials. The degree to which this is true depends on how well the cleaned material is dispersed prior to subsample withdrawal, and how evenly the withdrawn material is dispersed on the coverslip. Great care should be taken to ensure that these two steps are completed properly.

3.4.5 References

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3.5 Analysis of Diatoms

The purpose of the diatom subsample analysis is to estimate the proportion of diatom taxa found in a count of 600 valves (one-half of an individual diatom cell). On average, analysis of a slide should take approximately two hours; in no case should it exceed four hours or six transects. Biovolume measurements and imaging are part of this count process and should be completed within the four hour time frame. This does not include time spent learning new taxa when analyzing the first few samples in a new study unit. Images should be taken of all taxa encountered, including unknowns. Images need to be of sufficient quality as to be used for identification. Photos should be taken of all taxa listed on the taxa list over all of the samples.

3.5.1 Recommended Apparatus/ Equipment

- Compound microscope
- Oil immersion objective (100x) with a numerical aperture of at least 1.3
- Eyepieces of 10-15x
- DIC (differential interference contrast) or bright field condenser
- Diamond scribe mounted on microscope's objective stage
- High intensity light source

3.5.2 Methods

3.5.2.1 Diatom counts.

1. Scan slides at low to medium magnification (100x to 450x) to confirm that diatoms are evenly distributed on the coverslip and are at a density appropriate for efficient counting. At high magnification (1000x), there should be 10-20 diatoms per field. If there are problems with dispersion or density that would compromise the quality and accuracy of the analysis, discuss these with Diatom Preparation Lab personnel and have new slides made. Avoid counting diatoms in any disrupted areas of the mount, particularly edges that have optical aberrations. If diatoms on the slides are very sparse, refer to procedures in Section 3.4 for

handling low-density samples. Always save any count data generated for a sample, even if the number of valves or frustules is low (e.g., <100).

2. Because slides may need to be recounted for QA/QC purposes, it is very important to clearly demarcate the areas of a slide scanned during a count. After the preliminary slide examination, secure the slide in the mechanical stage and use the microscope's diamond scribe to etch a horizontal or vertical line (depending on personal preference) on the coverslip to mark the edge of the first row to be counted. Etch at least three lines on the diatom slide (if etching before counting) or a line for each row counted (if etched during an analysis). Rows are narrow rectangular areas (strips) of the slide adjacent to the scribed line, with width equal to the field of view. Start rows far enough from the coverslip edge to avoid optical distortion, and end them near the opposite coverslip edge where diatoms are no longer clearly visible (see diagram below). Locate a starting point near one end of the etched line and make a circle with the scribe. This denotes the starting point of the count. During the count, etch a circle around the last field counted in the first row and at the beginning and end of all other rows. Always check to make sure that etching is clearly visible so that circles and lines can be located easily by others.

3. When the line and first field are etched on the coverslip, and the first field is focused under oil immersion, begin the count. Record the transect start coordinates from the microscope stage.

4. Count diatom valves along transects, which are narrow rectangular areas (strips) of the slide with width equal to the field of view. The transect width should be the maximum width common to all participating taxonomists (at least 90 microns)

5. Count 600 valves. Count all partial valves that are more than 50% of the valve or that contain unique features such as recognizable central areas or distinct ends. Count all valves and fragments that extend at least halfway into the transect. Record the transect end coordinates from the microscope stage.

3.5.2.2 Biovolume measurements.

Biovolumes should be determined for all taxa either by measurements of cells observed in the project samples, from databases with known sources, or from the literature. The following criteria should apply regardless of the source of measurements.

Biovolumes for each abundant taxon (i.e., occurring in more that 5% in any one sample) should be based on measurements of 10 cells or more.

Biovolumes for each common taxon (i.e., occurring 2-5% in any one sample) should be based on measurements of one cell or more.

Biovolumes for each rare taxon (i.e., occurring in 0.1-2% in any one sample) should be based on measurements from literature descriptions of taxa or measurements of one cell or more.

For each taxon requiring biovolume measurements, select a simple geometric figure matching the shape of the taxon as best as possible, and determine the dimensions that must be measured. Average biovolume for a taxon should be calculated as the average of biovolumes of cells measured versus the average dimensions of cells measured. When a dimension of a cell is not known (e.g. depth), it should be estimated based on sizes of cells of the same taxon with similar known dimensions. Depth can be estimated by focusing on tops and bottoms of cells if other cells of the same taxon can not be found for measure of unknown dimensions.

The calculation of phytoplankton abundance depends on the apparatus used during analysis. Biovolume values are determined by multiplying the abundance (cells/ml) by the average biovolume of each cell (μm^3). Phytoplankton abundance (cells/ml) is calculated as follows:

$$cells/ml = \frac{count \times chamber\_area \times sample\_volume \times subsampleDCF}{trans\_length \times trans\_width \times chamber\_volume}$$

where count = number of cells counted, fieldDCF = field dilution/concentration factor, chamber = chamber area (in mm^2), subsampleDCF = subsample dilution/concentration factor, field = microscope field area (in mm^2), numfields = number of microscope fields, and chambervol = chamber volume used.

3.5.3 Quality Assurance/ Quality Control

Sample and slide quality can affect the outcome of these procedures. Minor deviations that do not affect the area scanned or number of specimens observed should be described on bench sheets. Other deviations should be discussed with the Project Manager for inclusion in the project QA/QC notes. A total of 10% of the samples collected from each study unit will be analyzed for quality control. EPA will identify which random slides will be sent to an independent laboratory for re-counting. Refer to Section 3.10.

3.5.4 References

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3.6 Analysis of All Algae

This protocol describes a quantitative procedure for analyzing all algae in samples. This procedure is semi-quantitative (i.e. describing cells per sample, per unit substrate area sampled, or per unit water volume sampled) and designed to provide data on algal densities and amount of algal biovolume. This protocol describes procedures for identification, counting, documentation, and measurement of algae. Often this protocol is referred to as the soft-algal count, which refers to the emphasis on identification of non-diatom algae.

This protocol also describes a tiered analysis of all algae. The simplest protocol is the 300 natural unit count, which was used in NAWQA, EMAP, and NLA programs.
With the exception of time spent learning new floras, the counting of 300 natural units should be finished in approximately two hours, on average.

3.6.1 Definitions

Natural counting unit. Algae grow unicellularly or in multicellular filaments and colonies. Each natural grouping of algae (i.e., each individual filament, colony, or isolated cell) is defined as a natural counting unit. Diatoms are an exception; each diatom cell is always considered a natural counting unit, even if attached to other cells. The main purpose of using 'natural counting units' is to prevent a colonial or filamentous form from dominating a count. It also facilitates the counting of algal forms which have linked cells that may be hard to distinguish.

Palmer-Maloney Count. This count type is to be completed on all samples. A maximum volume of 0.1 ml of the soft algal subsample viewed in two Palmer-Maloney cells will be assessed.

Using a transect approach, soft algae and live diatoms are enumerated until 300 natural counting units are reached or a total transect length of 115.7 mm (for field of view 0.54 mm) is assessed. Dead diatoms are also enumerated.

Dilution liquid. Samples collected as part of many sampling programs, including NAWQA, are preserved with a 2-3% formalin solution. Dilution liquid refers to the 3-5% formalin solution added to fractions when it is necessary to make dilutions of these fractions.

Macroalgae. A soft-algae component that consists of visible colonies or filaments.

Microalgae. A soft-algae component that consists of forms which are not visible without the aid of a microscope.

3.6.2 Recommended Apparatus/ Equipment

- Compound microscope with 20x objective for a total system magnification of 200x and 40-45x objectives for a total system magnification of 400-450x.
- Glass microscope coverslips, rectangular, 26 x 60 mm, #1 thickness.
- Glass, wide-bore pipettes > 1 mm inside diameter, or eye-dropper.
- Two Palmer-Maloney Counting Cells with ceramic chamber (chamber depth of 0.4 mm; volume of 0.1 ml) or metal chambers
- Glass pasteur pipettes, 5.25 inch, ≤ 1 mm diameter.
- Glycerin

3.6.3 Methods

Palmer-Maloney count

1. Place a rectangular cover slip (#1 thickness, 22 x 50 mm) at 45^{o} to the counting cell, covering about 1/3rd of the chamber, but not across the center of the cell.

2. Thoroughly mix the Palmer-Maloney fraction and draw into the micro-pipette 100 µl (plus suitable additional amount to allow for displacement from the chamber by the cover slip and prevent the occurrence of air bubbles). Quickly add the fraction drop-wise, into the center of the chamber. When the surface tension starts to draw the cover slip across the chamber, adjust the sides of the cover slip so that ends of the chamber are covered and the cover slip hangs over both sides of the ceramic portion of the counting cell.

3. Add glycerin to the area where the cover slip extends past the ceramic portion. This seals the cover slip to the counting cell temporarily (without excess heat or vibration, the counting cell can be used for a week or more).

Palmer-Maloney Fractions.

4. Dilutions or concentrations of samples with extremely high or extremely low diatom abundance may be necessary. This must be considered on a case by case basis. In general 15 – 30 natural counting units (both diatom and soft algae) should be visible per field of view. Assessment of five fields of view is advised. For samples where the number exceeds 30 natural counting units, dilution is recommended. Where the number is < 15, consider concentration. Silt/detritus levels are also a factor. In samples with high silt/detritus levels, even if there are < 15 natural units per field of view, consider dilution. Avoid concentrating samples were the silt/detritus level would be raised to a level too high to complete a successful count. Note on the count sheet when silt/detritus levels prohibit concentration or lead to a dilution. Both Palmer-Maloney cells should be prepared using the same Palmer-Maloney fraction. The S-R count should be completed on the original sub-sample taken from the total collected material. Only concentrate/dilute a portion (10 ml or less) of the original sub-sample. No sample should be diluted or concentrated to more than 20 % of its original volume.

   Note: For samples with high amounts of sand and/or silt, place the aliquot in the Palmer-Maloney Cell for assessment. Dilute if necessary and recheck sample. If large inorganic particles are present (but NOT organic detritus) and blocking view of algal cells, again thoroughly mix the Palmer-Maloney fraction. Allow the sample to stand while slowly counting to 5 or 6 (approximately 4-5 seconds). Re-load the Palmer-Maloney cell with the subsample according to instructions and count the sample or dilute again as necessary. This procedure is valid ONLY for samples with large amounts of inorganic sand and silt. No settling should occur with samples that contain organic detritus or at any time during the dilution process.

   Once the Palmer-Maloney cell is prepared, allow chamber to stand for up to 10 minutes to allow contents to settle.

5. Palmer-Maloney fraction data should be recorded on a separate log and the sheet included with the deliverables.

Enumerate 300 natural counting units.

6. Position the microscope stage so that the first field of view is in the top left hand area of the Palmer-Maloney cell at, but not overlapping, the cell edge. Using transects, of varying length, with a width of 0.54 mm (or width of the field of view) scan up to, but not exceeding, a total transect length of 115.7 mm (or equivalent). Every other transect, moving down the Palmer-Maloney cell, is to be enumerated, i.e. each transect enumerated should be

separated by a width of one field of view. This will prevent field of view overlap. For microscopes where the 40x field of view differs from 0.54 mm calculate the maximum transect length required. The full length of each transect should be scanned for algal enumeration. Record the start and end coordinates for each transect for the purpose of total transect length calculation.

7. Identify and enumerate all soft algal forms within the field of view. Algal forms are enumerated using algal natural units and identified to the lowest possible taxonomic level.

8. The taxa names to be used for this project, when identifying soft algal forms, are listed in the NRSA 2008 start list for soft algae [amended at the 2008 NAWQA soft algal workshop to the current accepted taxonomy]. This is to aid with taxonomic harmonization. This list helps promote consistency in identifications among laboratories.

9. Diatoms are differentiated as to “living” or “dead” at the time of collection. If there is any protoplast material in the frustules the diatom is considered to have been living when collected.

10. Count the number of algal cells for each multicellular natural unit. When cell length within a filament cannot be determined then a standard length of 10 µm per cell should be used. Notes should be made on the count report when this standard length is employed.

11. The number of cells for multicellular natural units is recorded in parenthesis beside the tally of natural counting units. All diatoms are grouped into one category – undifferentiated diatoms.

12. Tally data onto a bench sheet or into a computer based program. Notes on difficulties encountered in taxa identification due to the use of Lugol’s should be made during the count and logged with the count data.

13. Repeat procedures until 300 natural counting units have been enumerated. Only “living” diatoms are counted against the needed 300 natural algal units. “Dead” diatoms are recorded separately to inform the proportion of living vs dead diatoms in the sample.

14. Digital images are required for all identified taxa using the criteria outline in the Images section of this document (Section 3.9.1).

15. Biovolume measurements are required for all identified taxa using the criteria outline in the Biovolumes section of this document (Section 3.6.4).

16. Once 300 natural counting units have been enumerated, or the total transect length has been assessed, cease counting and record the total length of transects scanned.

3.6.4 Calculations and Reporting

The calculation of algal cell density and biovolume can be made per sample, per unit area of substratum sampled, or per volume of water sampled for qualitative and quantitative analyses of benthic and planktonic algae. Cell abundance per sample (cells/sample) is calculated as follows:

Equation 1

$$cells/sample = \frac{count \times chamber\_area \times sample\_volume \times subsampleD\,CF}{trans\_length \times trans\_width \times chamber\_volume}$$

where *count* = number of cells counted, *chamber_area* = chamber area (in mm^2), *sample_volume*=total volume (ml) of sample from which subsamples were drawn, *subsampleDCF* = subsample dilution/concentration factor, *trans_length* and *trans_width* are the length (mm) and width (mm) of transects counted, and *chamber_volume* = chamber volume used.

The *subsampleDCF* = subsample dilution/concentration factor is calculated by dividing the final diluted or concentrated subsample volume by the initial volume. So, a 10 mL subsample concentrated to 1 mL would have a *subsampleDCF* =0.1. A 10 mL subsample diluted to 100 mL would have a subsampleDCF=10.0.

Currently, there are not plans to calculate cells per unit area or volume of samples collected in the NWCA. If there were, cells per unit area or volume sampled can be determined by dividing the cells/sample by the area or volume sampled. If the laboratory sample (i.e. *sample_volume*) is a subsample of a larger sample collected in the field, then the laboratory sample fraction of the total field sample should be taken into account to determine the area or volume sampled corresponding to the laboratory sample.

Biovolume values are determined by multiplying the abundance (e.g. cells/sample) by the average biovolume of each taxon (μm^3).

Reports should be presented in excel spread sheets or comma delimited files. A template will be provided that includes rows for: sample ID, site ID, site visit number, date sampled, full taxon name, number of cells of the taxon counted, number of cells of the taxon per sample, and

biovolume of the taxon counted. Divide number of valves by two to calculate diatom cell abundance. Separate files should be prepared for the diatom and all-algae counts.

3.6.5 Quality Assurance/ Quality Control

It is critical that prior to taking a small portion of the subsample, the sample be thoroughly mixed. A small portion of the subsamples from each group of samples is re-processed using final dilution/concentration factors and checked for number of natural units per microscope field. Minor deviations to this protocol are expected, especially concerning methods to break up algal clumps. These deviations should be reported to project managers and/or algal taxonomists and noted on the Data Form. About 10% of the sample vials will be swapped and sent to an independent laboratory for a QA/QC analysis. Refer to Section 3.10.

3.6.6 References

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3.7 Phytoplankton Chlorophyll a

Chlorophyll-*a* samples are filtered in the field, placed in a labeled vial, and stored on ice until arrival at the laboratory. The laboratory should store the filter in the freezer at –20°C ±2°C for no more than 3 ½ weeks before analysis.

In addition to specifying laboratory methods for sample analysis, we use a performance-based approach that defines a set of laboratory method performance requirements for data quality. Method performance requirements for this project identify detection limit, precision, and accuracy objectives for each parameter (Table 3.7-1).

Table 3.7-1. Performance Requirements for Chlorophyll *a* Analytical Methods.

Analyte	Units	Potential Range of Samples[1]	Long-Term MDL Objective[2]	Laboratory Reporting Limit[3]	Transition Value[4]	Precision Objective[5]	Bias Objective[6]
chlorophyll *a*	µg/L (in extract)	0.7 to 11,000	0.5	1.0	15	± 0.5 or ±10%	± 0.5 or ±10%

[1] Estimated from samples analyzed at the WED-Corvallis laboratory between 1999 and 2005 for TIME, EMAP-West, and WSA streams from across the U.S.

[2] The long-term method detection limit is determined (eq. 1a) as a one-sided 99% confidence interval from repeated measurements of a low-level standard across several calibration curves, and includes medium or mean method blank results, (USGS Open File Report 99-193, EPA 2004). These represent values that should be achievable by multiple labs analyzing samples over extended periods with comparable (but not necessarily identical) methods.

[3] The minimum reporting limit is the lowest value that needs to be quantified (as opposed to just detected), and represents the value of the lowest nonzero calibration standard used. It is set to 2x the long-term detection limit/ fractional spike recovery, following USGS Open File Report 99-193 and EPA 2004.

[4] Value at which performance objectives for precision and bias switch from absolute (≤ transition value) to relative 9> transition value). Two-tiered approach based on Hunt, D.T.E. and A.L. Wilson. 1986. *The Chemical Analysis of Water: General Principles and Techniques*. 2nd ed.. Royal Society of Chemistry, London, England.

[5] For duplicate samples, precision is estimated as the pooled standard deviation (calculated as the root-mean square) of all samples at the lower concentration range, and as the pooled percent relative standard deviation of all samples at the higher concentration range. For standard samples, precision is estimated as the standard deviation of repeated measurements across batches at the lower concentration range, and as percent relative standard deviation of repeated measurements across batches at the higher concentration range.

[6] Bias (systematic error) is estimated as the difference between the mean measured value and the target value of a performance evaluation and/or internal reference samples at the lower concentration range measured across sample batches, and as the percent difference at the higher concentration range.

3.7.1 Summary of Method

Chl-*a* containing phytoplankton in a measured volume of sample water, are concentrated by filtering at low vacuum through a glass microfiber filter under low light conditions. The filter is then placed into a labeled centrifuge tube and frozen at -20° C. The pigments are extracted from the phytoplankton in 90% acetone with the aid of a mechanical tissue grinder. The filter slurry is allowed to steep for a minimum of two hours to ensure thorough extraction of the chl-*a*. The sample is centrifuged for 15 minutes at 675 x gravity. An aliquot of the supernatant is transferred to a glass cuvette and fluorescence is measured on the Turner Fluorometer. The concentration of chl-*a* in samples is determined from the calibrated instrument reading and from dilutions as necessary. The concentration of chl-*a* in the natural water sample is reported in µg/L. This SOP also includes chl-*a* standard preparation and calibration of the fluorometer.

3.7.2 Definitions

Laboratory Blank: An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with samples. The laboratory blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Primary Calibration Standard: A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Standards and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

RO water: Water passed through a reverse osmosis system.

SSCS: Secondary Source Check Standard is a sample containing the analyte of interest at known concentrations. The SSCS is obtained from a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials prepared independently from the normal preparation process.

3.7.3 Interferences

After the filters are ground the samples are cloudy and filter particles are suspended in solution. The samples must be clarified by centrifugation prior to analysis to prevent interference.

Contamination that fluoresces in the red region of the light spectrum may interfere in the accurate measurement of chl-*a*.

Interference by chlorophylls *b* and *c* are avoided by the use of the Turner Optical Filter Kit 10-040. Thus, acidification of the sample followed by measuring fluorescence of the acidified sample is not necessary for this method.

Minimum sensitivity settings on the fluorometer should be avoided due to quenching effects in highly concentrated solutions. Dilutions should be performed instead.

Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. All standards and samples must be at the same relative temperature during analysis.

Photosynthetic pigments are light and temperature sensitive. All work must be conducted in low light and samples must be stored in the dark at -20° C to prevent degradation.

3.7.4 Safety Precautions

This method does not address all safety issues associated with its use. The laboratory staff is responsible for safely conducting lab work and chemical analysis in accordance with the NHEERL-WED Health and Safety Handbook, the applicable Dynamac Hazardous Activity Safety Plans, and the Material Safety Data Sheets for the specific chemicals.

Proper personal protection equipment (PPE) is worn to prevent exposure to organic solvents.

Measures need to be taken to minimize exposure to acetone fumes. Proper room ventilation and use of a chemical fume hood is required for all procedures involving acetone. Consult the MSDS for detailed safety and handling information for acetone

3.7.5 Equipment and Supplies

- Model 10-AU Digital Turner Fluorometer equipped with: 1) 13-mm cuvette holder; 2) Optical Filter Kit PN 10-040, which includes: Blue lamp (PN 10-089); Emission Filter (PN 10-115); (436 nm) Excitation Filter (PN 10-113); (1 ND) Reference Filter (PN 10-032)
- Centrifuge, capable of 675 x gravity
- Tissue grinder, PowerGen 125
- Whatman GF/F glass fiber filters
- Centrifuge tubes, polypropylene, 50-mL capacity with screw caps

- Tweezers or flat tipped forceps
- Vacuum pump capable of maintaining a vacuum of 6 in. Hg (20 kPa)
- Room thermometer
- 13 x 100 mm Borosilicate glass culture tubes (10 mL)
- 10-mL pipette and disposable tips
- Graduated cylinders (various sizes as needed)
- Safety glasses
- Nitrile gloves
- Lab coat
- Laboratory exhaust fume hood
- Analytical balance with resolution to 0.01 mg
- Filter apparatus
- 50-mL volumetric flasks
- Explosion proof refrigerator
- -20º C Freezer
- Spectrophotometer, UV-visible, located in EPA Main Building Room 246.

3.7.6 Reagents and Standards

Reagents

1. Acetone, reagent grade

2. Chlorophyll *a* free of chlorophyll *b*. (Sigma-Aldrich; P.O. Box 14508; St. Louis, MO 63178; 800-325-5832. C6144 from algae, C5753 from spinach. Purchased yearly.)

3. RO Water: Water passed through a reverse-osmosis system to produce water similar to ASTM Type I reagent with 16.7 megaohms resistivity (Reference 16.5).

4. Primary and Secondary Calibration Standard Preparation

5. Gently tap the contents of the chl-*a* standard ampoule/vial to the bottom of the ampoule/vial.

6. Break or open the ampoule/vial. Transfer 1-3 mL of 90% acetone to the ampoule/vial. Using a Pasteur pipette, carefully transfer the contents to a 50-mL volumetric flask. All glassware must be clean and acid-free before use. Thoroughly rinse the ampoule/vial (including sides) a second, then a third time with 90% acetone transferring the contents each time to the 50-ml volumetric flask.

7. Fill the volumetric flask to volume using 90% acetone.

8. Measure the chl-*a* primary standard using a UV-visible spectrophotometer. Verify the measured value of chl-*a* against its calculated concentration.

9. After measuring absorbance at 750, 664, 647, and 630 nm, the concentration of chl-*a* in the 90% acetone standard may be calculated by the following equation, which corrects for absorbance at 750 nm (simplified):

chl-*a* (mg/L) = 11.85 (Abs 664) – 1.54 (Abs 647) – 0.08 (Abs 630) – 13.31 (Abs 750)

10. Remake the standard if the chl-*a* concentration is outside 5% of expected value. If this is not feasible, perform repeat measurements until a stock standard concentration can be confidently assigned.

11. Prepare the secondary source check standard (SSCS) in the same manner as above using an alternate source of chl-*a* (see Section 7.1.2).

12. Store all stock solutions in an explosion proof refrigerator at 4° C in a dark container.

Caution*: Acetone is hazardous to your health and is a highly flammable material. Do not allow skin contact or inhale vapors. Acetone rapidly degrades PVC. Wear gloves constructed of resistant material and work in a chemical fume hood.*

Preparation of Working Standards

1. Prepare working standards of chl-*a* using the primary standard solution. The dilutions are prepared in 90% acetone to the final concentrations of 0, 2, 20, 50, 100 and 200 µL chl a/L.

2. Working standards are stored in an explosion proof refrigerator at 4° C in a dark container.

3.7.7 Sample Collection, Preservation and Storage

1. Filtering should be performed in subdued light as soon as possible after sampling. The applied vacuum should be monitored with a gauge or manometer to ensure filtration pressure does not exceed 6 in. Hg (20 kPa). Higher filtration pressures may damage cells and result in a loss of chl-*a*.

2. Thoroughly shake the container to suspend particulates before the sample is poured for filtering. Accurately measure 500 mL of sample. Remove the filter from the base with

tweezers, fold once and insert inside screw cap centrifuge tube. Cover the outside of the tube with foil for added protection from light.

3. Sample filters are stored frozen (-20° C) in the dark until extraction.

4. Samples can be stored up to 4 weeks before extracting (Reference 16.1).

3.7.8 Calibration and Standardization

Calibration of the Fluorometer

1. Allow the fluorometer to warm up for at least 15 minutes.

2. Calibrate the fluorometer using the 50 µg chl-*a*/L primary calibration standard. Make sure to note the room temperature when recording calibration data.

3. Measure the fluorescence of each standard at the sensitivity setting that provides a midscale reading.

4. Record all measurements and analyze the linearity of the curve to ensure r>0.999.

5. Calibration is performed before each analysis, or when there has been an adjustment made to the instrument such as replacement of lamps, filters or the photomultiplier. The instrument should also be recalibrated if the room temperature fluctuates ±3° C from the initial calibration temperature.

Calibration Verification

1. Measure the 50 µL chl-*a*/L SSCS after calibration.

2. Record the SSCS measurement on the datasheet (Section 17.0).

3. Continue to measure the SSCS and a laboratory blank every 10 samples. If SSCS drifts outside ±10% recovery, recalibrate.

3.7.9 Procedure

Sample Preparation

1. Set up the filtration apparatus in the lowest light possible to prevent degradation of chl-*a* in the samples. The filter apparatus should be clean and acid-free.

2. Set-up a standard laboratory vacuum apparatus using a trap to collect excess/overflow water preventing aspiration into the vacuum system/pump.

3. Remove the samples from low-light containers and shake thoroughly to suspend the particulates. Carefully measure a 500-mL aliquot of the sample, and filter it through a Whatman GF/F glass microfiber filter. Vacuum filtration must not exceed 6 in. Hg (20 kPa). Higher vacuums may damage cells and result in loss of chl-*a*. Important note: Don't allow the filter to stay on the filter apparatus too long. The vacuum should be turned off at the valve when the sample is filtered so as to protect the chl-*a* in the sample.

4. Remove the filter using forceps, fold it in half, and place into a centrifuge tube labeled with an identification number. Centrifuge tubes should be placed in a lightproof secondary container before storage.

5. Store the filter in a freezer at -20° C for at least 24 hours, to lyse the cells and release the chl-*a* contained within them. The samples should be removed from the freezer and analyzed within approximately 25 days.

Extraction of Filter Samples

1. Remove the samples from the freezer. Maintain low light throughout the extraction.

2. Pipette 40 mL of 90% acetone into each centrifuge tube.

3. The filters are ground completely using the PowerGen 125 tissue homogenizer. Rinse the homogenizer thoroughly with RO water between samples to avoid cross-contamination. Small pieces of filter remaining on the homogenizer need not be transferred into the sample solution.

4. Vortex each tube to resuspend particulates within the solution.

5. Samples should be allowed to steep between 2-24 hours in an explosion proof refrigerator at 4° C.

6. After steeping is complete, vortex the sample again to resuspend particulates.

7. Centrifuge samples for 15 minutes at 675 x gravity. Allow samples to come to ambient temperature before analysis. Check and record room temperature before analysis.

Caution*: Acetone is hazardous to your health and is a highly flammable material. Do not allow skin contact or inhale vapors. Acetone rapidly degrades PVC. Wear gloves constructed of resistant material and work in a chemical fume hood.*

Sample Analysis

1. Allow the fluorometer to warm up for at least 15 minutes.

2. Use a 90% acetone solution to zero the instrument on the sensitivity setting that will be used for sample analysis.

3. Read the SSCS in the mid-range of the calibration that is pertinent to the sample range.

4. Transfer 8 mL of the sample into a borosilicate culture tube. Care should be used not to disturb the solids at the bottom of the tube during the transfer.

5. Choose a sensitivity setting that yields a midscale reading when possible and avoid the minimum sensitivity setting. If the concentration of chl-*a* in the sample is too high to be measured, dilute the sample to the appropriate midscale range using 90% acetone.

6. Record the fluorescence measurement and dilution data if applicable on the data sheet (see Section 17.0).

3.7.10 Data Analysis and Calculations

To calculate the concentration factor for chl-*a*, enter measurements into spreadsheet template, which uses the equation:

Concentration factor = extraction solution volume (mL)/sample volume (mL)

Raw data are reported by electronic spreadsheet in µg/L, and this data is then used to calculate chl-*a* using the following calculations:

(1) Cf/a = (extraction solution volume/filtered volume)*(total volume/area scraped)

(2) Chl-*a* = raw data (µg/L) * Cf/a (mL/cm_2) * (1L/1000mL)

Where:
Extraction solution volume = 40 mL (unless noted)
Filtered volume = volume of sample filtered (usually 25 mL)
Total volume = volume of sample collected (usually 500 mL)
Area scraped = (# of transects)*(area delimiter (cm^2))
Cf/a = concentration factor per area (see above)

Duplicate precision is determined from analytical results.

Report results to three significant figures.

3.7.11 Method Performance

Method performance is measured through analysis of blank measurements and duplicate precision. Blank measurements should be <0.015 µg/L. These measurements ensure accuracy at low-level samples. Duplicate precision must be within 10%. On occasion duplicates will fall outside this range; this variance is most likely a result of lack of homogeneity in the prefiltered sample solution. The possible reasons for imprecise duplicate measurements include lack of homogeneity, evaporation of acetone, and instrument drift, and should be noted on the datasheet. If duplicates are not in good agreement and there is no apparent reason or observable differences, the instrument should be recalibrated and samples reanalyzed.

3.7.12 Pollution Prevention

1. The chemicals used in this method pose little threat to the environment when properly managed.

2. All standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of waste. Wastes are collected for recycling or appropriate disposal.

3.7.13 Waste Management

1. It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, and to protect the environment by minimizing and controlling

all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is required.

2. Contact the Contractor Program Health and Safety Manager for guidance on waste collection and disposal. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel*, and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, DC 20036.

3.7.14 Quality Control

Analytical Duplicate is a separate analysis from the same sample aliquot, run a minimum of once every 10 samples. The analytical duplicate is the same aliquot run near the end of the run.

Blank is a reagent blank taken from the 90% acetone solution and measured as a sample. A blank should be analyzed every 10 samples.

SSCS is mid-range in calibration and is measured every 10 samples. Recalibrate if outside 10% of the expected value.

Room temperature should be monitored and the instrument recalibrated if the room temperature varies ±3° C of the initial calibration.

Personnel performing this procedure are trained and must demonstrate their ability to handle standards in normal laboratory conditions without significant photodegradation. Stock solutions and working standards must routinely be transported through lighted hallways; during these times they should be well protected against the fluorescent lighting, which is particularly harmful to chlorophyll pigments. New analysts will demonstrate the ability to adequately light-proof standards before transporting them through lighted areas.

3.7.15 References

"A Procedure for Measuring Extracted Chlorophyll *a* Free from the Errors Associated with Chlorophyll *b* and Pheopigments", Turner Designs Method. 2006.

Arar, E.J. and Collins, G.B. "Method 445.0: *In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence", U.S. EPA

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Arar, E. J. "Method 446.0, rev 1.2: *In Vitro* Determination of Chlorophylls a, b, c_1 + c_2 and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry". U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH, 45268. 1997.

ASTM. American Society for Testing and Materials. Standard Specifications for Reagent Water. D1193-77 (Reapproved 1983). Annual Book of ASTM Standards, Vol. 11.01. ASTM: Philadelphia, Pennsylvania, 1991.

Welschmeyer, N.A. 1994. "Fluorometric Analysis of Chlorophyll *a* in the Presence of Chlorophyll *b* and Pheopigments", Limnology and Oceanography, 39:1985-1992.

3.8 Microtiter Plate Enzyme-Linked Immuno-Sorbent Assay (ALISA) for Microcystin

This section describes the process for performing Microtiter Plate ELISA of Microcystins using the Abraxis Polyclonal ADDA kit at the USGS Organic Geochemistry Research Laboratory (OGRL) in Lawrence, KS. Results are for water samples and concentrations are reported between 0.10µg/L and 5.0µg/L without dilution. This method is suitable for water and algae samples that have been lysed and/or filtered.

3.8.1 Definitions

Laboratory Duplicate- An identical sample; designated here by a letter extension, L, appended to the log number.

ELISA- Enzyme-Linked Immunosorbent Assay.

IMN- Immunoassay for Microcystin analysis code.

Immunoassay Quality Assurance Sheet- Also called "the blue sheet." This is a sheet for each project analyzed that has a list of samples analyzed that day. It is also used to track data from the analysis, through data entry, through QA, and to the Project Manager's office to be sent out.

Data Entry Sheet- This is a sheet generated from Excel that provides the needed information for the data entry person to be able to enter the concentrations into the database used to store, retrieve, and print reports for all project data.

Project Quality Control Sample- Each project that is analyzed IMN analysis must have a QC sample that contains a hit of microcystin from that project (so actual representative matrix of that project). This QC sample is re-analyzed every time a sample from that project receives IMN analysis.

Laboratory Spike- An identical sample; designated here by a letter extension, S, appended to the log number. The final concentration will be 0.75µg/L of Microcystin-LR plus the ambient sample concentration.

3.8.2 Safety Precautions

This SOP is to be used in conjunction with an approved Chemical Hygiene Plan. Also, consult the Chemical Hygiene Plan for information on and use of all PPE.

The Stopping Solution consists of a weak acid. Do not allow it get on your clothes or yourself. Wash the acid off immediately with copious amount of water.

3.8.3 Equipment and Supplies

Descriptions of commonly used pieces of equipment, their advantages and their limitations are listed below.

- Samples: the Project Manager will provide a list of samples to be analyzed. They should be located in Mac Shared to Lab.
- Project Quality Control Samples: These are designated by the person performing this SOP as new projects come in. The samples are stored in the immunoassay refrigerator door for easy access. The data from this sample is reviewed by the Quality Assurance checker.
- Microcystins Plate Kit (Abraxis): A commercially available kit that contains the antibody coated microtiter plate and the reagents needed to perform the assay. A set of instructions is included in the kit.
- Multichannel Pipette & Tips: An 8-channel pipette is used for this method. Familiarity of the use of the multichannel pipette is necessary to achieve reliable results. Practice with water if you have never used this before.
- Reagent Reservoirs (Costar Cat Number 4870): Plain plastic reservoir for reagents that accommodate the use of a multi-channel pipette.

- 100 µL Pipette and Tips: For measuring and transferring standards, controls, and samples into the antibody coated plates.
- 1000 µL Pipette and Tips: For diluting samples for reruns.
- Vortex Genie: For mixing dilutions.
- Adhesive Sealing Film (Parafilm) for Micro Plates (such as Rainin, non-sterile, Cat. No. 96-SP-100): Used to cover plates during incubation.
- Orbital Shaker Table (such as American Shaker Table V, Model R4140): To be used for mixing microtiter plates during incubations.
- Permanent Marker (Sharpie Fine Point): For labeling samples, bottles, plates and covers.
- Timer: For measuring incubation times.
- Distilled Deionized Water: For diluting samples.
- Paper Towels: For blotting the microtiter plates dry after washing.
- Plate Reader (such as Metertech, Model M965 AccuReader): Complete with Metertech PC Mate software for operation of machine. This machine reads the microtiter plates.
- Computer: To operate plate reader and record data.
- Printer: To print the results.
- Immunoassay Quality Assurance Sheet (blue sheet): Found on ResLab under Immunoassay Data. This is used by the QA checker and will be written on when results are printed off.
- Data Entry Sheet: Found on ResLab under Immunoassay Data. This is where results are input for the data entry person.
- Three Ring Notebook: For storage of data, found in room 313 on bookshelf.
- Test tubes: For dilutions, if needed.
- Log-in Records Binder: This contains the hard copies of log-in information used for checking the accuracy of log-in against the labels on the sample bottles. This binder is stored in the Project Manager's office (room 313) or in the lab by the plate reader.

3.8.4 Procedures

Note: Deviations from SOPs must be recorded in an appropriate instrument or work log.

Allow Immunoassay kits and samples to warm to room temperature before analyzing (about one hour). Make certain there is enough of all the reagents to complete the number of analyses before beginning, if not allow another kit to warm.

Preparation following the manufacturer's instructions:

1. Samples will undergo a total of three freeze-thaw cycles. For the first cycle, thaw (at room temperature) the 250ml glass ambers bottles the samples were collected in. Aliquoted approximately 10ml of each well-mixed sample into new, labeled 20ml glass scintillation

vials, one per sample. Placed the 20ml scintillation vials in a freezer to complete the two additional freeze-thaw cycles, for a total of three freeze-thaw cycles. All thaw cycles should be completed at room temperature. After the last frze-thaw cycle, filter approximately 10 ml of each sample through a new, syringe filter (0.45µm) into a new, labeled 20ml glass scintillation vial. (Norm-ject syringes and Whatman Glass Fiber syringe filters (25mm, GF 0.45µm filter, catalog #6894-2504) or other similar alternative are acceptable.) One new syringe and filter should be used per sample.

2. All immunoassays are categorized by project code and then numerical order; therefore, separate the samples by project code first and include a QC sample for each project code. For each set of 10 samples, the first and fifth samples are duplicates. One spiked sample should be included for every regular sample.

3. On the ResLab, prepare the microcystin data entry sheet by entering the QC samples and samples, date analyzed, project code, etc.

4. The pipettor, tips, sealing film, pens, test tubes, and reagent reservoirs are located in the immunoassay drawers.

5. Using the PC Mate software, enter into the template, the location of the standards, controls, and samples on the Microtiter Plate. The plate can be made up of rows of strips of 8 wells that will snap into a blank frame. Label each row with a pen.

6. Analyze all five standards (0.00, 0.15, 0.40, 1.00, and 5.00) in duplicate. Space the standards at the beginning.

7. The PC Mate software is on the immunoassay computer and is used for controlling the microtiter plate reader and for calculating results. There is a manual for this software, and it should be read before using the program.

8. Prepare the appropriate template and print it for reference for loading the standards and samples. At this time enter plate name, file name, operator, etc. and save all the information. File names include the date the assay was performed and includes the letter of the assay. For example, since the analysis code for Microcystin is IMN, we use the N from IMN at the end of the file name. Before the N, the seq name order should be year, month, and then day. If the day was 1/20/05, the file name would be 05JAN20N.

9. Turn on the plate reader using the switch on the back to allow it to warm for at least 30 minutes. The plate reader may need to be turned on before the computer boots up so that the computer can access and control the plate reader.

Assay Procedure (There are also manufacturer instructions in the kit contents. All procedures are in agreement with the manufacturer's instructions.):

10. Prepare spiked samples as called for on run sheet by adding 15 µL of a 25 µg/L microcystin-LR standard solution to 500 µL of sample in a labeled LC vial. Cap and vortex. (Note on 25 µg/L microcystin-LR standard solution: This solution does not need to be made fresh daily. Stock m microcystin-LR standard can be made by dilution in LC/MS grade methanol. Be sure to check purity by UV-VIS extinction coefficient or LC/MS/MS. It is acceptable to make batches from the stock in glass 2 mL LC vials and freeze them half-full. The final methanol content is 5% or less in the 25 µg/L MCLR standard used for ELISA.)

11. Using the 100 µL pipette, add 50µl of the standards, controls, samples and spiked samples (prepared in 6.3.1) to the appropriate wells in the plate as indicated by the IMN run sheet.

12. Immediately add 50µL of the pink antibody solution to each well using the multi-channel pipette and a reagent reservoir.

13. Place Sealing Parafilm over the wells.

14. Place the plate on the orbital shaker table. Protect tray from light, and set the speed for 180 rpm and the timer for an hour and a half.

15. Carefully remove the Parafilm.

16. Empty the plate into the sink, pat dry with a stack of paper towels, and then wash the wells of the plate 3 times with 250ul of washing solution using the multi-channel pipette. Each time you add the washing solution, let the washing solution set about 45 seconds before emptying it into the sink and using the paper towels as before.

17. Add 100µl of enzyme conjugate solution to all wells using the multi-channel pipettor.

18. Cover the wells with Parafilm and place on the orbital shaker table in a dark corner at 180-rpm for 30 minutes.

19. After 30 minutes, rinse the wells 3 times again with 250ul of washing solution.

20. Add 100µl of substrate solution to the wells using the multi-channel pipette and reagent reservoir, and incubate on the orbital shaker at 180 rpm for 25 minutes. Cover with Parafilm and protect from light. This will make the contents have a blue hue.

21. Uncover and add 50ul of stopping solution to the wells. This will turn the contents a bright yellow color. After you have added the stopping solution, the plate must be read within 15 minutes using the plate reader.

Reading the Plate

22. Change the settings in the PC Mate program if needed to agree with the manufacturer's instructions. The current settings for the Microcystin Assay are:
    - Wavelength 450nm
    - Curve is: 4-parameter fit
    - Display: Analyzed
    - Endpoint: L1, Auto mix: on, cal:on, disk:on, print:OFF

Reporting Data

23. The software calculates the values of the samples from the Calibration Curve, and averages the two results. Print this report out.

24. Reporting limits for this procedure range between 0.10 µg/L to 5.0 µg/L. If a dilution is performed, higher concentrations can be reported. Non-detections are reported as "<0.10 µg/L".

25. Prepare the Data Entry Sheet if you have not already.

26. There is an Excel workbook in ResLab labeled Immunoassay Data. Copy a previous sheet and type today's information into it, including Lab ID, project code, concentrations to report (get this information from the raw data print out), analysis date, any remarks, your initials as the tech ID, etc. The entire plate's samples go on a single Date Entry Sheet (i.e.: it can have multiple projects on it).

27. An example of the Data Entry Sheet is shown in Attachment C.

28. When typing in the concentration of the sample, you will find that there are three values on the strip. Use the value that says the mean concentration, the last value of the three. This will be input into your data entry sheet. The strip will show if it is "HI" for out of range values.

29. Samples needing dilutions because of "HI" concentrations do need QA's approval and can be redone in the next run. The same is true for any other redo, such as if the duplicates did not agree, you ran out of stop solution, or there was a bad standard curve.

30. For non-detects (flagged as "nd") list as less than the reporting limit ("<0.10").

31. You should also enter “DO NOT ENTER” for samples that had high concentrations, needed dilutions, or ran out of reagent, as well as for QC samples. QC values go in a separate data base.

32. To determine if the control is correct, type in the found value in the analyzed column (in blue font at the bottom of the page). It will calculate a percent difference which must be within 20% of the control concentration.

33. Print out the sheet and attach it to the blue sheet.

34. Print out the computer information and attach it to the blue sheet (include all calibration curves and values).

35. Complete the Immunoassay Quality Assurance Sheet (i.e. the blue sheet).

36. This is in the same Excel workbook as the data entry sheet. Copy over the previous sheet with new information. Prepare a new blue sheet for each project analyzed on this plate. You must type in the project code, analysis code (IMN), plate name, analysis date, and the lab ID’s. Print the sheet(s) out on blue paper, which is kept in drawer LciC11&12 with the other colored paper.

37. Do a bottle check (a verification of the log-in accuracy) on the samples analyzed.

38. Obtain the 3-ring binder of Log-In records from the Project Manager’s office (Rm 313).

39. Using the Lab ID’s (MM#’s) to guide you, compare the information printed in the binder to what is on the bottle and look for discrepancies. Check the project code, site name, station ID, date collected, and time collected.

40. Place a check mark (✓) next to the Lab ID in the binder that corresponds to the bottle you just verified to signify that the log-in information has been checked against the bottle.

41. If you find a mistake or discrepancy, write in the correction in the binder, initial your correction(s) in the binder, immediately report it to the Project Manager, and see that it gets corrected in the project’s database computer.

42. Initial and date the Immunoassay Quality Assurance Sheet (blue sheet) to indicate that sample labels have been verified against log-in and corrections made.

43. An example of the Immunoassay Quality Assurance Sheet is shown in Attachment B.

44. You now have a data set consisting of the raw data (plate template, concentrations, and standard curve) printed from SoftMax, the Data Entry Sheet, and the Immunoassay Quality Assurance Sheet. Paperclip these together with the Immunoassay Quality Assurance Sheet on top and turn it in to the Data Entry box for IM analyses (Rm 309).

45. Dispose of solution in plates in a lab sink. Rinse the plate and sink with water to dilute the weak acid present.

46. All data from the analysis is put in the IMN folder, which is found in the Project Manager's office. That report includes the Curve that is printed out from the computer, the data sheet, and the template for the standards and the samples. Staple this information together.

Dilutions if needed are prepared as follows (using clean disposable plastic tubes):

47. 1. 1:10 dilution
    - Pipette 100µl from the sample in to the tube.
    - Add 900 µl of distilled water to sample above. (Note: Dilutions may also be made using the kit's dilutenet rather than distilled water.)
    - Mix by Vortexing.
    - Multiply final concentration by 10.

48. 1:100 dilution.
    - Pipette 10µl from the sample.
    - Add 990µl of distilled water to sample. (Note: Dilutions may also be made using the kit's dilutenet rather than distilled water.)
    - Mix by Vortexing.
    - Multiply final concentration by 100.

49. Other dilutions can be calculated if needed.

3.8.5 Quality Control

The Quality Assurance Officer or designee will evaluate overall data quality and QC compliance. In the event data is not in compliance, the problem(s) will be identified and samples will be reanalyzed as appropriate after corrective action taken.

The standard curve should have a correlation coefficient of 0.99 (as suggested by ELISA kit manufacturer).

The absorbency of the blank must be >1.400 (as suggested by ELISA kit manufacturer).
The Check Standard supplied with the ELISA kit should be analyzed a minimum of two times in each run, once at the beginning and once at the end. This helps ensure the plate was prepared in the proper time frame. Values should be +/- 20% (28.3% relative standard deviation (RSD)) of expected value.

Laboratory duplicates should have a percent Relative Standard Deviation (% RSD) of 28.3 percent or less when compared to each other (as suggested by ELISA kit manufacturer).
If laboratory duplicates are outside of this range, then they should be reanalyzed in the next run.

Quality control samples are available for each project. Criteria for acceptance of measured values are +/- 20% of average. These samples are analyzed everytime samples from the same project code are analyzed.

A designated archived project sample (**Quality Control Project Samples)** is reanalyzed with every run set that is analyzed. Control charts are maintained for these samples. A running historical average is maintained of the concentration from each run. The concentration of the QC sample for each successive run has to be ± 20 percent of that average to be acceptable.

3.8.6 References

USGS. 2010. Standard Operating Procedure: Microtiter Plate Enzyme-Linked Immuno-Sorbent Assay for Microcystin. OGRL-SOP-5630.

3.9 Taxonomic Standards Protocols

The taxonomic nomenclature for the NWCA will follow previous lists that have been developed for the 2008 National Lakes Assessment, the National Rivers and Streams Assessment, and the USGS National Water-Quality Assessment (NAWQA) Program list (Appendix B). These three lists have not been reconciled but the names used are sufficiently consistent. These lists include accepted names for all distinct taxa for both known and unknown species. The laboratory is responsible for reconciling all identifications with these taxa lists. Voucher images are reviewed regularly to ensure accuracy and consistency among taxonomists.

If a species of algae cannot be conclusively determined, then several strategies can be used to characterize what is observed.

1. If a taxon is observed repeatedly and can be consistently distinguished from other diatoms, then it is given a Counter Taxon Name designation in the following format:
   Unknown Species
   a. Genus name
   b. "X" is a unique sequence number for unknown species in that genus
   c. NWCA 11 is the unique project identifier
   d. "YYY" are the initials of the taxonomist who first made the identification
   e. Example species name:
      i. *Nitzschia* sp. X NWCA11 YYY
      ii. *Nitzschia* sp. 1 NWCA11 EAR
      iii. *Nitzschia* sp. 2 NWCA11 EAR
      iv. *Acanthosphaera* sp. 1 NWCA11 EAR
      v. *Cyanobacterium* sp. 1 NWCA11 EAR

The Counter Taxon Name designation can also be used at higher levels of taxonomy in the following format:

Unknown Genus

a. "Unknown" identifies the species as having an unknown genus
b. Division name (i.e., Rhodophyta or Chlorophyta)
c. Brief description of algae to help taxonomists distinguish between unknowns
d. "X" is a unique sequence number of unknown species of that division
e. NWCA11 is the unique project identifier
f. "YYY" are the initials of the taxonomist who first made the identification
g. Example species name:
   i. Unknown Chlorophyta coccoid sp. X NWCA11 YYY
   ii. Unknown Chlorophyta coccoid sp. 1 NWCA11 EAR
   iii. Unknown Rhodophyta flagellate sp. 1 NWCA11 EAR

2. If a taxon looks similar to another taxon, but not sufficiently to be identified as that taxon, then the "cf" designation is used in the following format:
   a. Genus name
   b. "cf" designates the taxon as not sufficiently identified
   c. Species name
   d. Last name of the taxonomist to first use the designation in capital letters
   e. Species authority
   f. Example species name:
      i. *Chroococcus* cf. *minimus* CIUGULEA (Keissler) Lemmermann

3. If a taxon was only observed in one sample and can be distinguished from other algae in the sample, but it is not tracked in other samples or cannot be related to another taxon, then it is

assigned as “sp. X ?”, where X is a unique sequence number. This designation can be used with genus or higher levels of taxonomy, e.g. Unknown Cyanophyte sp. 1?

4. If a specimen cannot be identified to species level or distinguished clearly from other unknowns in a sample, then the designation “sp.” or “spp.” is used. The designation “sp.” is used when there is likely only one taxon of undistinguishable cells in the sample (e.g., *Dictyosphaerium* sp.). The designation “spp.” is used when there is likely more than one taxon of undistinguishable cells in the sample (e.g., *Dictyosphaerium* spp.). These designations can also be used at higher levels of taxonomy, but it is important to include cell shapes or growth forms so that taxonomists can distinguish between unknowns, e.g. Unknown Chlorophyte coccoid (>10µ) GDL or Unknown Chrysophyte flagellate.

Girdle views of diatoms are often difficult to identify to species level. However, taxonomists for the NWCA samples should identify diatoms in girdle view to species and variety level based on their knowledge of the taxon in valve view and their ability to understand the three dimensional morphology of a specimen.

3.9.1 Images

Taking pictures, illustrating taxa, and maintaining an image database is important for consistent identification of taxa by individuals and groups of taxonomists. The NWCA protocol requires good quality digital images to be supplied for all taxa that represent 5% or greater abundance of any one sample. Good quality means that the taxon can be identified under the microsope from that digital image. Unknown taxa also must be imaged and shared with other taxonomists. Images of all unknown taxa given NWCA species designations shall be provided to document these taxa. Images shall be shared among taxonomists so all taxa, named species, and NWCA unknowns, can be consistently identified in samples by more than one taxonomist. These images are critical for increasing consistency in taxonomy, i.e. ensuring that the same name is used for commonly occurring ‘unknowns’ throughout a project.

Images should be taken at a focal depth at which most key diagnostic features are distinguishable and clear. Flat specimens are preferred over tilted specimens. They should be taken at the magnification that the samples were counted. Images should be cropped to include only one taxon. The image should include a scale bar or measurements to indicate the size of the specimen. Consistent labeling of images is helpful when maintaining an image database. The naming convention used for images in the NWCA image database shall be as follows:

Taxa name_magnification (if applicable)_sample ID.xxx

e.g. Homoeothrix janthina_40x1.5x_NWCA0001.jpg

3.9.2 Table of References

Documentation of the concepts used for all taxa is important. If it is not practical to take a good digital image of certain taxa, the taxonomist should provide an image from a reference that best represents the morphological concept of the taxon as used in the study. The taxonomist is responsible for developing a NWCA Reference Table, which lists all of the NWCA Taxa Names, references used to identify the taxa, and an image from a plate in the literature or collection of digital images. Literature and image references will be provided for all taxa with NWCA Taxa Names at the species or variety level and all NWCA unknown taxa that are category 1 unknowns, e.g. "*Ankistrodesmus* sp. 1 NWCA JAH." Each species identified at the species or variety level with a species name or identified as a distinct unknown is incorporated in the reference table.

3.9.3 Taxonomic Harmonization

Successful taxonomic harmonization among taxonomists is essential for accurate data analysis. Inconsistent identifications of algae introduce error in ecological analyses, so reducing this source of error is important. Harmonization of identifications among taxonomists is particularly important in large projects in which more than one taxonomist identified algae. The process of harmonization can be accomplished without limiting the taxonomic explorations of taxonomists. To accomplish both goals, the lab develops harmonization tables in which names used in identification by each taxonomist are related to names that other taxonomists used in the project.

Taxonomists will participate in conference calls as needed or at least once a month to harmonize taxa for known diatoms and soft algae. Unknown species are also reconciled to a standard naming convention during this time. During these conference calls, taxonomists share species images for 1) all unknown taxa given Counter Taxon Names, and 2) known species that occupy 5% or great of either count.

During the harmonization process, names assigned by taxonomists during counting, referred to as Counter Taxon Names, are assigned NWCA Taxon Names based on taxonomist agreement. For known taxa the Counter Taxon Name and the NWCA Taxon Name may be the same. However, in some instances a NWCA Taxon Name may correspond to one or more Counter Taxon Names, (i.e., multiple taxonomists recognize the same unknown taxa). For unknown taxa name harmonization use the following standard naming convention:

NWCA Taxon naming convention for unknown species:

a. Genus name
b. "X" is a unique sequence number of unknown species of the unknown genus; note this number sequence restarts during the taxonomic harmonization process. For example, if taxonomist A's first Nitzschia species and taxonomist B's twelfth Nitzschia species are the same, then they would become the first harmonized Nitzschia species (i.e, Nitzschia sp. 1 NWCA11 EAR and Nitzschia sp. 12 NWCA11 MES become Nitzschia sp. 1 NWCA11 LAB).
c. NWCA11 is the unique project identifier
d. Name of lab identifies that the unknown species has been reconciled during the inter lab taxonomic harmonization process

NWCA taxa that are likely too similar for multiple taxonomists to name consistently are assigned a Lump Level 1 Name. Lumping similar taxa will reduce taxonomic variability and reduce the risk of error in the ecological analyses. This process is repeated to create a Lump Level 2 Name, which represents the coarsest level of species level taxonomy recommended for use in ecological analyses. With each level of harmonization, reasons for lumping at successively higher levels should be provided.

The product of the harmonization process is a Taxonomic Harmonization Table that provides the following information:

1. Counter Taxon Name
2. Reason for lumping Counter Taxon Name to an Lab Taxon Name, if it was lumped
3. NWCA Taxon Name
4. Reason for lumping NWCA Taxon Name to a Lump Level 1 Name, if it was lumped
5. Lump Level 1 Name
6. Reason for lumping Lump Level 1 Name to a Lump Level 2 Name, if it was lumped
7. Lump Level 2 Name
8. Comment

3.9.4 Final Products

The lab is responsible for sending the following data files to the Information Management Coordinator at NARSDataSubmission@epa.gov for the purpose of quality assurance: count tables in comma delimited text files or Access database tables, taxonomist reports, and harmonization (translation) tables. A written report detailing the methods used and discussing problems encountered must be included as an appendix to this report. In addition to these data files, the lab will send the reconciled and harmonized species list as a final product. All products should be provided in the same format as the National Rivers and Streams Assessment products.

3.10 Pertinent QA/QC Procedures

The QA/QC procedures for algae include taxonomic harmonization and re-counts of 10% of samples collected. These procedures should occur continuously throughout the project to correct for inaccuracies and ensure the final products contain high quality data. For re-counts of samples, analysts will swap vials of material and hard mounted slides for diatoms and recount following sample protocols. Re-counts will be performed by an inter-lab exchange. EPA will inform the laboratories which random samples will be re-counted. The samples must then be sent from the original laboratory to another laboratory. The QC taxonomist should complete another copy of the Taxonomic Bench Sheet for each sample. Each bench sheet should be labeled with the term "QC Re-ID." As each bench sheet is completed, it should be emailed to the Information Management Coordinator at NARSDataSubmission@epa.gov.

The lab will compare the taxonomic results generated by the primary and QC taxonomists for each sample based on both the raw data and the appropriate metrics (i.e., taxa identified with similar autecologies). EPA will then calculate percent similarity (Algae Chapter of QAPP). It is expected that the soft algae counts should have a similarity of ≥50% and the diatom counts should have a similarity of ≥70%. If not, the reasons for the discrepancies between taxonomists should be discussed. Results less than these values will be investigated and logged for indication of error patterns or trends.

A report or technical memorandum will be prepared by the QC Taxonomist. This document will quantify both aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report will be submitted to the Information Management Coordinator, with copies sent to the primary and QC taxonomists and another copy maintained in the project file. Significant differences may result in the re-identification of samples by the primary taxonomist and a second QC check by the secondary taxonomist. All samples must be stored at the laboratory until the project officer notifies the lab.

4. VEGETATION

4.1 Introduction to Indicator

Collectively, wetland plant species 1) represent diverse adaptations, ecological tolerances, and life history strategies, and 2) effectively integrate environmental conditions, species interactions, and human-caused disturbance. Data describing species composition and abundance and vegetation structure are powerful, robust, and relatively easy to gather (USEPA, 2002). Categories of data to be collected include the following:

1. Species composition and abundance
2. Alien species
3. Floristic quality
4. Guild composition
5. Community composition
6. Vegetation structure and productivity

For more detailed information on the utility of vegetation for assessing wetland condition please see *Ecological Indicators for the 2011 National Wetland Condition Assessment* (in preparation).

4.2 Receiving Voucher Samples

Processing and managing plant samples or identifying unknown species in the field is often very difficult and time consuming. Accordingly, it can be logistically infeasible to complete, along with vegetation sampling tasks, in a single day. Thus, it is extremely important that the Vegetation Team is prepared to ship samples to a herbarium or bring them back to the office to complete this task. Plant samples will arrive at the herbarium dried and pressed in shipping boxes.

4.2.1 Definitions

For the NWCA, a **voucher sample** is a pressed and dried plant sample, ideally comprised of leaves, stems, flowers, fruits and roots. An integral component of each voucher sample is written data describing the location, date of collection, habitat, plant habit, characteristic features, and other information. Vouchers provide physical evidence that confirms the presence of plant species at specific locations. Other uses for vouchers include 1) identifying species encountered and not immediately recognized by a botanist or other field workers during

ecological studies, 2) documenting the occurrence of native rare plants, or 3) cataloging the distribution of invasive alien plants.

For all NWCA field work, whenever the identity of a species cannot be confirmed in the field, a sample is collected (see Vegetation Chapter of Field Operations Manual (FOM)) for later identification in the office or lab. All **unknown species** in each Vegetation Plot (five 100-m^2 Veg Plots/AA) are collected. If an unknown species is collected at a previous site, it is collected at subsequent sites, until the Botanist/Ecologist learns the identity of the species and can reliably sight recognize it in the field. This is particularly important for species in difficult wetland genera and families, such as those that include sedges, rushes, grasses, and submerged aquatic vegetation. The Botanist/Ecologist will ship unknown samples to the Herbarium for identification (Vegetation Chapter of FOM).

In addition, one **known species** is randomly collected from each Vegetation Plot as quality assurance **voucher samples** (Vegetation Chapter of FOM). Collecting voucher samples of known species both provides a quality assurance check on species identity data, and a permanent record of the occurrence of a particular species at a given location. The field Botanist/Ecologist will ship known samples collected for quality assurance to the QA Herbarium.

One of the primary goals of the national survey is to build state and tribal capacity to implement wetland monitoring and assessment programs; therefore, EPA encourages states to participate in the vegetation portion of the lab manual wherever possible. For the purposes of this manual a **Herbarium** represents the person identifying and processing unknown samples. This could be a field Botanist/Ecologist, state identified herbarium, EPA identified regional herbarium, or National EPA Contractor. The Herbarium is responsible for ensuring all plant identification and processing tasks outlined in this manual are completed. In some cases this may require the Herbarium to identify partners to assist with the work.

Voucher samples collected for quality assurance will be sent to the designated **QA Herbarium**. A QA Herbarium is a certified botanist, state or EPA identified herbarium that agrees to use the NWCA prescribed methods, as described in Section 4.7 of this chapter, to ensure that all QA vouchers receive the same level of taxonomic precision. If the Herbarium and QA Herbarium are the same institution, it is important that all Quality Assurance activities are completed by another experienced taxonomist who did not participate in the identification of unknown species. The QA Herbarium will blindly re-identify all species to ensure that the identifications are independent.

See figure 4-1 for potential options for plant samples collected in each Vegetation Plot.

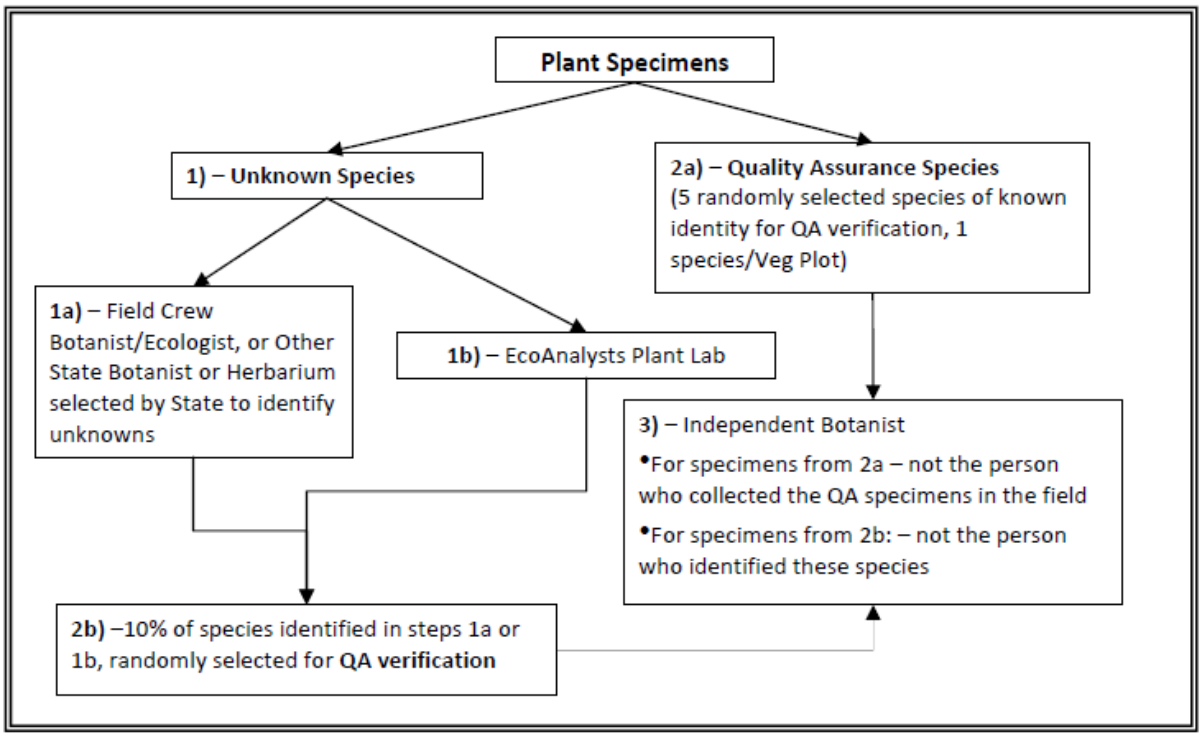
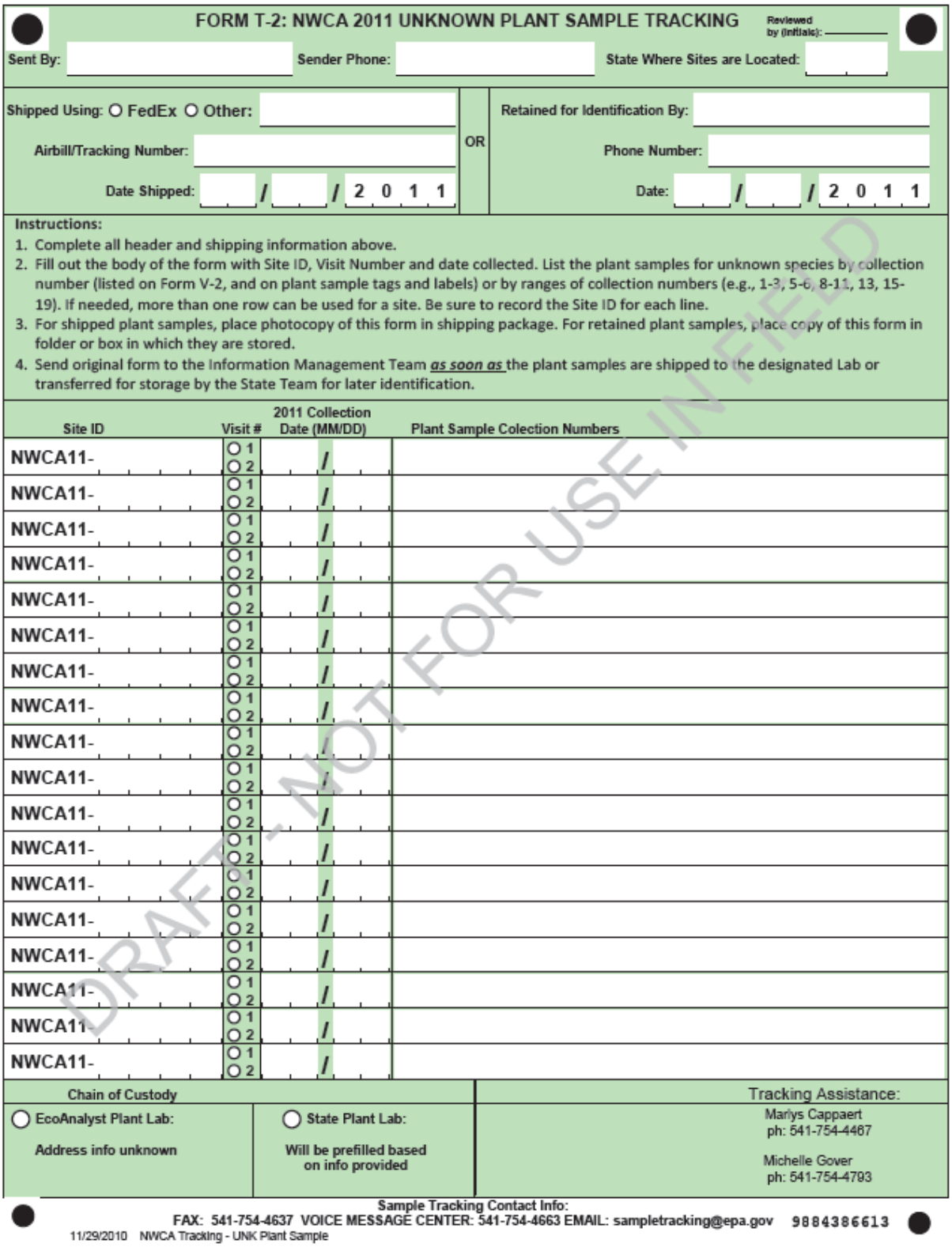


Figure 4-1: Potential options for plant vouchers. NWCA definitions for Herbarium and QA Herbarium are provided in section 4.2.1.

4.2.2 Tracking information

In the field, each voucher sample collected is assigned a set of tracking information, which is recorded on the Plant Sample Tracking Form (Figure 4-2a and 4-2b). At the end of the sampling week the vegetation team will remove the samples and newspaper sleeves from the press, ensuring they retain the Plant Sample Label Form (Figure 4-4), and ship them in a sturdy box to the Herbarium (Vegetation Chapter of FOM). If a sample listed on the tracking form is not part of the shipment, or a sample arrives at the lab without the proper label, contact the Information Management Coordinator (541-754-4663) as soon as possible.



FORM T-2: NWCA 2011 UNKNOWN PLANT SAMPLE TRACKING

Reviewed by (initials): _____

Sent By: _____ Sender Phone: _____ State Where Sites are Located: _____

Shipped Using: ○ FedEx ○ Other: _____

Airbill/Tracking Number: _____

Date Shipped: ___/___/2011

OR

Retained for Identification By: _____

Phone Number: _____

Date: ___/___/2011

Instructions:

1. Complete all header and shipping information above.
2. Fill out the body of the form with Site ID, Visit Number and date collected. List the plant samples for unknown species by collection number (listed on Form V-2, and on plant sample tags and labels) or by ranges of collection numbers (e.g., 1-3, 5-6, 8-11, 13, 15-19). If needed, more than one row can be used for a site. Be sure to record the Site ID for each line.
3. For shipped plant samples, place photocopy of this form in shipping package. For retained plant samples, place copy of this form in folder or box in which they are stored.
4. Send original form to the Information Management Team ***as soon as*** the plant samples are shipped to the designated Lab or transferred for storage by the State Team for later identification.

Site ID	Visit #	2011 Collection Date (MM/DD)	Plant Sample Colection Numbers
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	

Chain of Custody

○ EcoAnalyst Plant Lab: Address info unknown

○ State Plant Lab: Will be prefilled based on info provided

Tracking Assistance:

Marlys Cappaert
ph: 541-754-4467

Michelle Gover
ph: 541-754-4793

Sample Tracking Contact Info:
FAX: 541-754-4637 VOICE MESSAGE CENTER: 541-754-4663 EMAIL: sampletracking@epa.gov 9884386613

11/29/2010 NWCA Tracking - UNK Plant Sample

Figure 4-2a: Unknown Plant Sample Tracking Form

FORM T-3: NWCA 2011 QA PLANT SAMPLE TRACKING

Reviewed by (initials): _____

Sent By: _____ Sender Phone: _____ State Where Sites are Located: _____

Shipped Using: ○ FedEx ○ Other: _____ OR Retained for Identification By: _____

Airbill/Tracking Number: _____ Phone Number: _____

Date Shipped: ___/___/2011 Date: ___/___/2011

Instructions:

1. Complete all header and shipping information above.
2. Fill out the body of the form with Site ID, Visit Number and date collected. List the QA plant samples for species identified in the field and/or the QA samples for plant species identified in the Lab by collection number (listed on Form V-2 and on plant sample tags and labels). Collection numbers can be listed individually (e.g., 4, 6, 10) or as ranges (7-12) if appropriate. If needed, more than one row can be used for a site.
3. For shipped plant samples, place photocopy of this form in shipping package. For retained plant samples, place copy of this form in folder or box in which they are stored.
4. Send original form to the Information Management Team *as soon as* the plant samples are shipped to the designated Lab or transferred for storage by the State Team for later identification to allow proper tracking of plant samples.

Site ID	Visit #	2011 Collection Date (MM/DD)	Plant Sample Colection Numbers
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	
NWCA11-	○ 1 ○ 2	/	

Chain of Custody

○ EcoAnalyst Plant Lab: Address info unknown

○ State Plant Lab: Will be prefilled based on info provided

Tracking Assistance:

Marlys Cappaert
ph: 541-754-4467

Michelle Gover
ph: 541-754-4793

Sample Tracking Contact Info:
FAX: 541-754-4637 VOICE MESSAGE CENTER: 541-754-4663 EMAIL: sampletracking@epa.gov 0731469718

11/29/2010 NWCA Tracking - QA Plant Sample

Figure 4-2b: QA Plant Sample Tracking Form

4.3 Supplies and Equipment for Sample Handling

- Plant dryer
- Dissecting microscope
- Dissecting tools (e.g., single edge razor blades, forceps, dissecting needles)
- Regional floras and plant lists
- USDA PLANTS taxonomic standard (http://plants.usda.gov/java/)
- Plant nomenclatural forms
- Plant sample tracking forms
- Plant sample folders
- Storage cabinet or sealable plastic boxes for storing dried plant samples prior to identification
- OPTIONAL: Freezer or naphthalene for killing pests on dried plant material
- OPTIONAL: Mounting materials (herbarium sheets, mounting glue, forceps, weights for holding samples with wet glue to the herbarium sheets, etc)
- OPTIONAL: Herbarium sample labels

4.4 Handling Vegetation Samples

Plant samples may arrive at the Herbarium in several conditions: 1) as dried, pressed samples, or 2) pressed but still wet plant material enclosed in a plant press.

1. If samples are pressed and dried, proceed to Section 4.4.3 (Treat samples for detritivores, molds, and pests), then to Section 4.5 (Identifying Plant Samples).

2. If samples arrive in a press, but are still wet they should be placed on a plant dryer to complete drying, and then be treated for pests. This would likely occur if the Botanist/Ecologist is acting as the Herbarium.

4.4.1 Plant Sample ID Tag and Label Form

Every sample will arrive at the Herbarium with a Plant Specimen Label Form. This form includes diagnostic information for known and unknown species collected. Voucher samples are considered incomplete without this information. An example of the Plant Sample ID Tag Form is provided in Figure 4-3 and an example of the Plant Sample Label Form is provided in Figure 4-4. The Plant Sample ID Tag will be affixed to the outside of the newsprint in which each specimen is pressed. A duplicate tag will be affixed to the Plant Specimen Label. If a sample

does not have any of the following information, contact the Information Management Coordinator (541-754-4663) as soon as possible:

Plant Sample ID Tag Information

- **Plant Sample ID Number:** NWCA Site Number-Plant collection number. Plant collection numbers for samples are ordered chronologically for each site beginning with one. For example, the sample number for the 5th species collected at NWCA11-Point 0762 would be NWCA11-0762-5. Voucher samples collected for QA purposes will have the **QA** bubble filled.
- **Collection Date:** Date is numerical: month, day, year, e.g. 06/14/2011.

Plant Specimen Label Information

- **Plant Sample ID Number:** NWCA Site Number-Plant collection number. Plant collection numbers for samples are ordered chronologically for each site beginning with one. For example, the sample number for the 5th species collected at NWCA11-Point 0762 would be NWCA11-0762-5. Voucher samples collected for QA purposes will have the **QA** bubble filled.
- **Collection Date:** Date is numerical: month, day, year, e.g. 06/14/2011.
- **Pseudonym for Unknown Species:** Descriptive name used on data forms (e.g., Carex sp. 1). QA specimen will not have species name information on the label.
- **Collector(s) name:** List the first name, middle initial and surname of the person or persons who collected the sample.
- **Habitat**: The type of plant community or setting where the plant is growing. (e.g., such as wetland type (Cowardin, HGM, NVC), wetland community type (forested wetland, emergent marsh, wet prairie, mountain bog, etc.), anthropogenic disturbances (urban setting type), and, other plants growing in association (associated species information would be available from the plot).
- **Plant habit/description**: Describe key features of the plant such as growth form (tree, shrub, vine, herb), approximate height, longevity (annual, biennial, perennial), clonal, rhizomatous, tussock-forming, etc. List any characteristics of the plant which may be lost upon drying, such as flower/fruit color, fragrance, and leaf orientation.
- **Abundance of Plant**: Indicates whether the species is dominant, common, sparse or uncommon at the site, and whether based on canopy cover it is a dominant, moderate cover, or low cover species.

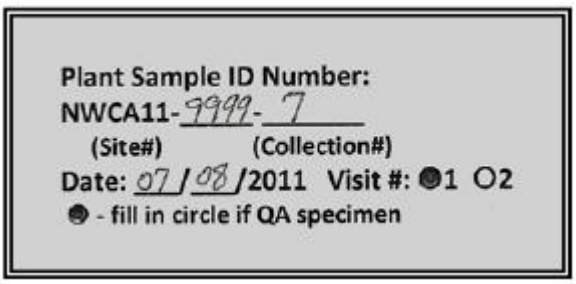
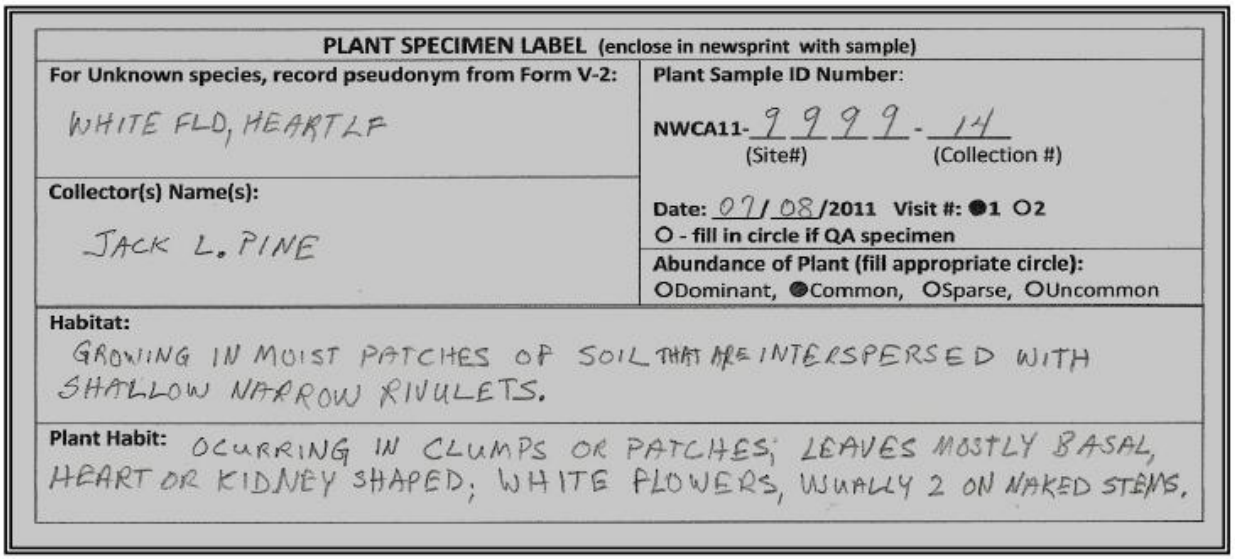

Plant Sample ID Number:
NWCA11-9999-7
(Site#) (Collection#)
Date: 07/08/2011 Visit #: ●1 O2
● - fill in circle if QA specimen

Figure 4-3: Example Plant Sample ID Tag


PLANT SPECIMEN LABEL (enclose in newsprint with sample)

For Unknown species, record pseudonym from Form V-2:
WHITE FLD, HEARTLF

Plant Sample ID Number:
NWCA11-9999-14
(Site#) (Collection #)

Collector(s) Name(s):
JACK L. PINE

Date: 07/08/2011 Visit #: ●1 O2
O - fill in circle if QA specimen

Abundance of Plant (fill appropriate circle):
ODominant, ●Common, OSparse, OUncommon

Habitat:
GROWING IN MOIST PATCHES OF SOIL THAT ARE INTERSPERSED WITH SHALLOW NARROW RIVULETS.

Plant Habit: OCURRING IN CLUMPS OR PATCHES; LEAVES MOSTLY BASAL, HEART OR KIDNEY SHAPED; WHITE FLOWERS, USUALLY 2 ON NAKED STEMS.

Figure 4-4: Example Plant Specimen Label

4.4.2 Drying Samples

Plant samples arriving at the Herbarium may still be wet and in the plant press (this would likely happen if the Botanist/Ecology is acting as the Herbarium). The pressed plants must be thoroughly dried before removing them from the presses. As the samples dry they will loose volume, so it is often necessary to periodically tighten the straps on the press to maintain pressure on the samples and minimize shrinkage and wrinkling.

Low ambient humidity and good airflow around and through the presses is important for rapid and thorough drying of plant material. Rapid drying over low heat promotes preservation of color and morphology resulting in high quality samples. Dry air circulating through the press also may

kill many insects and insect eggs, which may protect the samples from some insect damage. These conditions are most easily obtained by placing full presses on an electric plant dryer that provides steady bottom heat (95°F to 113°F), where plants usually dry in 12 to 48 hours. However, presses placed in a warm dry place will be sufficient if a plant dryer is not available.

4.4.3 Treat samples for detritivores, molds, and pests

Dried plant material is highly susceptible to contamination by detritivores, molds, and pests that can destroy herbaria collections. Therefore it is important to treat all incoming samples to kill potential contaminates.

Herbaria should implement their standard pest procedures. A common method for sample treatment is to freeze them (-20°C or below) for at least three days for loosely stacked samples and seven days for tightly packed samples.

To protect the collection from infestation, plant samples should be stored in herbarium cabinets or sealable plastic container when not in use; **under no circumstances** should samples be left out in the herbarium room overnight. If samples are found that have been left out overnight or if a cabinet/plastic container has been left open, all samples may need to be decontaminated again.

4.5 Identification of Vegetation Samples

4.5.1 Taxonomic standard

The recognition and identification of particular classes of plants such as families, genera, and species is a critical and difficult element of collecting accurate vegetation plot data. To complicate matters, not all botanical authorities agree about which name to apply to a particular plant species. The NWCA is using the taxonomic nomenclature of the USDA Plants Database as the taxonomic standard. To effectively key plants and identify them in the field, however, Field Crews may use local floras appropriate to each region or state (Appendix C). This means numerous taxonomies will likely be applied across the 48 conterminous states comprising the study area. The Herbarium will reconcile all species names that they identify to the standard found in USDA Plants.

4.5.2 Species Identifications for Voucher Samples

Once each plant specimen is identified, the taxonomist is responsible for reconciling the species name with the USDA Plants standard. All identifications are recorded in an excel database. Use the following steps to compare the final taxa list for each site to that of the USDA Plants website (http://plants.usda.gov/).

4.6 Mounting and Storing Herbarium Sheets

Once the samples are dried, pressed, and identified, they are to be stored at the Herbarium for at least five years. Vouchers should be kept in sealable plastic containers in a cool dry climate and must be accessible to the EPA. However, the Herbarium is encouraged to incorporate the NWCA vouchers into their permanent collections as desired. Vouchers from the national survey mounted on herbarium sheets should be labeled to indicate that they were collected as part of the NWCA. For an example of commonly used mounting and labeling methods see Appendix D.

4.7 Quality Assurance

A subset of plant samples collected as unknowns and later identified by the Herbarium will need to be verified by a QA taxonomist for additional Quality Assurance (QAPP section 5.1.8). The Herbarium will randomly select 10% of the samples to be sent to the QA taxonomist, another experienced taxonomist who did not participate in the original identifications.

Note: If the field botanist/ecologist is acting as the Herbarium then the QA Herbarium would need to be another qualified botanist, or a state or EPA identified herbarium. However, if a state or EPA identified herbarium is acting as the Herbarium then the QA activities could be completed by a taxonomist within the same institution that has not actively participated in the identification of the unknown species.

1. The QC taxonomist will perform re-identifications completing another copy of the Vegetation Taxonomic Bench Sheet for each sample. Each bench sheet must be labeled with the term “QC Re-ID.” As each bench sheet is completed, it must be faxed to the Information Management Coordinator.

2. The project facilitator will compare the taxonomic results generated by the primary and QC taxonomists for each sample and calculate percent taxonomic disagreement (PTD) as measures of taxonomic precision (Stribling et al. 2003) as follows:

Equation 1

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N}\right)\right] 100$$

where $comp_{pos}$ is the number of agreements (positive comparisons) and N is the total number of samples in the larger of the two counts.

3. Unless otherwise specified by project goals and objectives, the measurement quality objective for enumerations will be a mean PTD less than or equal to 15, calculated from all the samples in the 10% set sent to the QC taxonomist. Results greater than these values will be investigated and logged for indication of error patterns or trends, but all values will generally be considered acceptable for further analysis, unless the investigation reveals significant problems.

4. Corrective action will include determining problem areas (taxa) and consistent disagreements. Problems will be addressed through taxonomist interactions. Disagreements resulting from identification to a specific taxonomic level, creating the possibility to double-count “unique” or “distinct” taxa will also be rectified through corrective actions.

5. The project facilitator will prepare a report or technical memorandum. This document will quantify both aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report will be submitted to the project manager, with copies sent to the primary and QC taxonomists and another copy maintained in the project file.

4.8 References

Kansas State University. 2006. Integrated Pest Management Plan. http://www.k-state.edu/herbarium/pests.html. Accessed November 17, 2009.

Lee, M. T., R. K. Peet, S. D. Roberts, and T. R. Wentworth. 2008. CVS-EEP protocol for recording vegetation: All levels of plot sampling. Version 2008. The Carolina Vegetation Survey (CVS, http://cvs.bio.unc.edu) and the North Carolina Ecosystem Enhancement Program (EEP, http://www.nceep.net).

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Magee, T. K., S. E. Gwin, R. G. Gibson, C. C. Holland, J. E. Honea, P. W. Shaffer, J. C. Sifneos, and M. E. Kentula. 1993. Research Plan and Methods manual for the Oregon Wetlands Study. EPA/600/R-93/072. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oregon.

Stribling, J.B., S.R. Moulton, and G.T. Lester. 2003. Determining the quality of taxonomic data. Journal of the North American Benthological Society 22:621–631.

University of Florida Herbarium. 2009. Preparation of Plant Samples for Deposit as Herbarium Vouchers. http://www.flmnh.ufl.edu/herbarium/voucher.htm, Accessed June 12, 2009.

USEPA. 2002. Methods for Evaluating Wetland ConditionL #10 Using Vegetation to Assess Environmental Conditions in Wetlands. EPA-822-R-02-020, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2006. Survey of the Nation's Lakes. Laboratory Methods Manual. EPA841-B-06-005. U.S. Environmental Protection Agency, Washington, DC.

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5. SOIL

5.1 Introduction to Indicator

The presence of hydric soil is a defining characteristic of wetland ecosystems, and both influences and is influenced by hydrology and vegetation (Tiner 1999, Mitsch and Gosselink 2007). Categories of data to be collected include the following:

1. Hydric soil field indicators
2. Description of soil profile and physical characteristics
3. Soil chemistry
4. Soil disturbance

For more detailed information please see the report *Ecological Indicators for the 2011 National Wetland Condition Assessment* (in preparation).

5.1.1 References

Mitsch, W.J., and J.G. Gosselink. 2007. Wetlands. John Wiley & Sons, Hoboken, NJ.

Tiner, R. W. 1999. Wetland Indicators: A Guide to Wetland Identification, Delineation, Classification, and Mapping. Lewis Publishers, CRC Press, Boca Raton, FL, USA.

5.2 Quality Assurance/Quality Control

Standardized lab protocols, consistent training of all lab technicians, lab assistance visits to all labs, and availability of experienced technical personnel to respond to site-specific questions as they arise are important to ensuring the quality of lab data. Additionally, control measures to minimize measurement error among lab technicians and laboratories include the use of a Control Sample, a Blank Sample, Data Review, and Data Validation (QAPP section 5.5.6).

A **Control Sample** represents a sample of known concentration for a particular attribute. A Control Sample is collected in bulk for an attribute and repetitively analyzed to determine statistical control limits (i.e., range of expected values) for the particular method. A Control Sample is analyzed in conjunction with every batch of samples to ensure the method was run

correctly. If the value of the Control Sample falls outside the expected range of values then the process has failed and the batch is flagged for reanalysis.

A **Blank Sample** is used to ensure equipment is thoroughly cleaned before each use. A Blank Sample is especially important when measuring soil chemistry (i.e., trace metals) because concentrations may be quite small. A Blank Sample is analyzed in conjunction with every batch of samples to ensure that proper equipment cleaning protocols are followed. If the value of the Blank Sample does not equal zero or fall below the method detection limit, then the equipment is not clean and the batch is flagged for reanalysis.

The process of **Data Validation** is described here. Laboratory data undergo four **Data Reviews**, first by the Bench Analysts, second by the Lead Analyst, third by the Project Coordinator Soil Scientist, and fourth by a Soil Scientist Liaison with expertise in soils from the region where the samples are from. The Bench Analysts verifies that blank and control samples return results that fall within established control limits. The Lead Analyst examines the data for inconsistencies and apparent anomalies; inconsistencies usually take the form of unexpected high or low values for a particular analyte or values that do not fit with the expected trend of a soil profile. The Project Coordinator will use professional judgment to determine whether the project data are self-consistent and congruent with the site data collected in the field; incongruities within the data that can be explained either by site data or the results of other analytes are recorded. A final review is given by a Soil Scientist Liaison to the area of sample origin, before the data are released.

5.3 Receiving Regulated Soils

Soils that may contain pests (i.e., bacteria, plant viruses, fungi, nematodes, and life stages of destructive mollusks, acari, and insects) are regulated by U.S Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). Areas within states that are under Federal quarantine must follow the conditions and safeguards prescribed by APHIS before shipping to another part of the country. To ensure that the NWCA is in compliance with APHIS recommendations, all soils collected for the survey will be shipped as regulated soils. Participating labs are responsible for obtaining and maintaining a valid permit for receiving regulated soils (see example, USDA APHIS PPQ 525-A, Figure 5-1 below).

Upon arrival at the lab soil samples will be separated into regulated and non-regulated based on their county and state of origin (as recorded on the water proof label affixed to the outside of the sample bag). The lab is responsible for following all APHIS protocols when handling or disposing regulated soils as found in 7 CFR 330.300.

Animal and Plant Health Inspection Service

Plant Protection & Quarantine

United States Department of Agriculture
Animal and Plant Health Inspection Service
4700 River Road
Riverdale, MD 20737

Permit to Receive Soil

Regulated by 7 CFR 330

This permit was generated electronically via the ePermits system.

PERMITTEE NAME:	Dr. Thomas Reinsch	**PERMIT NUMBER:**	P330-08-00009
COMPANY:	USDA-NRCS-NSSC	**APPLICATION NUMBER:**	P525-071002-007
RECEIVING ADDRESS:	Federal Building, Room 152, MS 41 100 Centennial Mall North Lincoln, NE 68508-3866	**DATE ISSUED:**	01/14/2008
MAILING ADDRESS:	Federal Building, Room 152, MS 41 100 Centennial Mall North Lincoln, NE 68508-3866		
PHONE:	(402) 437-4179		
FAX:	(402) 437-5760	**EXPIRES:**	**01/14/2011**

PORTS OF ARRIVAL/PLANT INSPECTION STATIONS: Various Ports of Entry Staffed by CBP-Agriculture Inspection

HAND CARRY: No

Under the conditions specified, this permit authorizes the following:

Quantity of Soil per Shipment and Treatment
Over 3 lbs

PERMIT CONDITIONS

1. This permit authorizes the importation of soil, under the conditions specified below. Upon arrival in the United States, the articles, shipping container(s), and paperwork are subject to inspection by officials of Customs and Border Protection, Agriculture Inspection (CBP-AI) and the USDA, Plant Protection and Quarantine (PPQ).

2. Under the Plant Protection Act, individuals or corporations who fail to comply with the following conditions and authorizations, or who forge, counterfeit, or deface permits or shipping labels will receive civil or criminal penalties, and will have all current permits cancelled and future permit applications denied.

3. Any person who unloads, lands, or otherwise brings or moves into or through the United States any regulated plants, plant products, plant pests, soil or other products or articles in violation of the regulations will be subject to prosecution under the applicable provisions of the law.

Permit Number P330-08-00009

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Maria Corpuz	01/14/2008

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to $250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)

Animal and Plant Health Inspection Service

Plant Protection & Quarantine

4. All foreign cargo of agricultural interest is inspected at the first port of arrival or the first port of unlading. If a shipment arrives at a port without the required official personnel available to do the proper inspection, and/or treatment, any subsequent movement, or any transfer and/or transloading, must be approved by CBP-AI.

5. A copy of this permit must accompany all shipments authorized under this permit.

6. The soil is to be shipped in sturdy, leak-proof containers.

7. CBP-AI and PPQ have the option to order and approve treatment, re-exportation or destruction of a shipment, a portion of a shipment, or any other material associated with the shipment (i.e. pallets, packaging, means of conveyance). This will be done if the official personnel find that the shipment requires treatment as a condition of entry, is contaminated with a quarantine plant pest or pests, is commingled with prohibited plant material, or if required documentation is incomplete or missing.

8. The shipment must be free from foreign matter or debris, plants, noxious weed seeds, and living organisms such as parasitic plants, pathogens, insects, snails, and mites. Material found to be commingled with unauthorized material will be subject to the same action (i.e. re-export, destruction) as the unauthorized material.

9. All solid wood packing material (SWPM) present with this shipment must be in compliance with ISPM 15 treatment and IPPC stamp requirements and enforcement. Noncompliant shipments will be treated, re-exported or destroyed at the consignee's expense.

10. All costs and arrangements for the safeguarding of the cargo and the transportation of the cargo are the responsibility of the importer, broker, or other parties associated with the shipment.

11. The shipment can be released without treatment at the port of entry to the permittee's address listed on the permit or label, or an authorized user only if the final destination is an approved facility listed at https://web01.aphis.usda.gov/PPQ/AuthSoilLabs.nsf/web?openform.

12. Permit is to be utilized by the permittee or authorized user only (authorized users must present a written, dated, and signed statement on letterhead from the permittee, along with a valid ID and a copy of this permit).

13. There is no further distribution of soil without prior approval from the State and Federal Regulatory Officials. Soil is to be used strictly for analysis in a laboratory environment at USDA-NRCS-NSSC located in Lincoln, NE.

14. Upon receipt, all samples will remain within the approved soil laboratory identified on this permit. Laboratory access is restricted to individuals authorized by the permit holder.

15. This permit does not authorize the use of soil for growing purposes and or the isolation or culture of organisms sourced from imported soil.

16. All unconsumed soil, containers, and effluent is to be autoclaved, incinerated, or properly sterilized by the permittee at the conclusion of the project as approved and prescribed by PPQ in the compliance agreement.

17. Valid for shipments of soil not heat treated at the port of entry, only if a Compliance Agreement (PPQ Form 519) has been completed and signed. Compliance Agreements and Soil Permits are non-transferable. Notify local USDA office promptly if the permittee leaves the company.

18. This permit authorizes shipments from all foreign sources, including Guam, Hawaii, Puerto Rico, and the U.S. Virgin Islands through any U.S. port of entry.

END OF PERMIT CONDITIONS

Permit Number P330-08-00009

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Maria Corpuz	01/14/2008

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to $250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)

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Figure 5-1: USDA APHIS PPQ 525-A for receiving regulated soils.

5.4 Parameters for the NWCA

A total of 15 parameters will be measured from each soil sample collected (Table 5-1). Table 5-1 also lists example methods that have been demonstrated to achieve the required objectives.

Table 5-1: Soil parameters measured for the National Wetlands Condition Assessment.

Method	SSL Method	Analyte	Units	MDL	PQL	Reproducibility			Potential Sample Range	Accuracy Objective
						Value	N	RL		
PSDA, <2mm, air dry	3A1a1a	Clay	%	na	na	32	296	2	0 to 93.1	
		Silt	%	na	na	22	297	3	0.1 to 100	
		Sand	%	na	na	8.3	297	2	0 to 94.5	
Calcium carbonate equivalent, <2mm	4E1a1a1a1	$CaCO_3$	%	0.5	2.5	9.6	134	1	nd* to 105	
Calcium carbonate equivalent, <20mm	4E1a1a1a2	$CaCO_3$	%	0.5	2.5				nd to 96	
Total Carbon, Nitrogen and Sulfur	4H2a1-3	C	%	0.04	0.2	3.1	184	0.3	nd to 62.43	0.01%
		N	%	0.04	0.2	0.19	183	0.04	nd to 11.193	0.001%
		S	%	0.04	0.2	0.016	183	0.02	nd to 21.86	0.01%
pH	4C1a2a1a-b1	1:1 H_20	pH	na	na	6.1	129	0.3	2.4 to 10.5	0.1 pH unit
	4C1a2a2a-b1	1:2 0.01 M $CaCl_2$	pH	na	na	5.4	129	0.3	2.3 to 10.5	0.1 pH unit
Cation exchange capacity	4B1a1b1-4	CEC	cmol(+) kg^{-1}	0.1	0.6	26	75	2	nd to 252	0.1 cmol(+) kg-1
		Ca^{2+}	cmol(+) kg^{-1}	0.07	0.4	15	77	1	nd to 507.3	0.1 cmol(+) kg-1
		K^{+}	cmol(+) kg^{-1}	0.06	0.3	0	77	0.1	nd to 17.4	0.1 cmol(+) kg-1
		Mg^{2+}	cmol(+) kg^{-1}	0.01	0.07	9	77	0.6	nd to 147.1	0.1 cmol(+) kg-1
		Na^{+}	cmol(+) kg^{-1}	0.2	1.0	1	77	0.3	nd to 650.3	0.1 cmol(+) kg-1
Ammonium Oxalate Extraction	4G2a1a1-5	Al	%	0.002	0.009	1.1	102	0.2	nd to 15.62	0.01%
		Fe	%	0.0001	0.0006	0.6	102	0.08	nd to 20.15	0.01%
		Mn	mg/kg	0.1	0.6	296	102	42	nd to 15730.7	1.0 mg/kg-1

		P	mg/kg	26	129	867	102	234	nd to 16926.4	1.0 mg/kg-1
		Si	%	0.0002	0.001	0.41	102	0.05	nd to 6.13	0.01%
Electrical Conductivity	4F1a1a1a1	EC	mmhos cm^{-1}	0.001	0.005	3.1	17	0.3	nd to 167.4	0.01 mmhos cm-1
Dithionite-Citrate Extraction	4G1a1-3a-b1	Al	%	0.001	0.006	0.19	207	0.09	nd to 8.6	0.1%
		Fe	%	0.01	0.07	2.2	207	0.4	nd to 36.6	0.1%
		Mn	%	0.0006	0.003	0.091	207	0.03	nd to 3.6	0.1%
Olsen Phosphorus	4D5a1a-b1	P	mg/kg	0.1	0.7	13	32	3	nd to 399.7	0.1 mg/kg-1
Mehlich Phosphorus	4D6a1a-b1	P	mg/kg	0.1	0.6	19	12	2	nd to 1232.3	0.1 mg/kg-1
Trace Elements	4H1a1a1a1-20	Ag	mg/kg	0.001	0.01	1.8	126	0.7	nd to 175.62	
		As	mg/kg	0.002	0.01	50	126	8	nd to 1808.06	
		Ba	mg/kg	"0"	"0"	500	126	54	0.02 to 4415.8	
		Be	mg/kg	0.001	0.01	0.86	126	0.3	nd to 29.98	
		Cd	mg/kg	0.001	0.01	1.6	126	0.4	nd to 85.68	
		Co	mg/kg	"0"	"0"	10	126	1	nd to 1125.58	
		Cr	mg/kg	0.006	0.03	22	125	8	nd to 2020.31	
		Cu	mg/kg	0.002	0.01	78	126	7	nd to 1036.28	
		Hg	µg/kg	1.3	6.50	212	126	58	nd to 26060	
		Mn	mg/kg	0.002	0.01	1311	126	63	nd to 692942	
		Mo	mg/kg	0.001	0.01	na	na	na	nd to 235.17	
		Ni	mg/kg	0.009	0.05	139	126	17	tr* to 3347.36	
		P	mg/kg	0.4	2.07	949	126	106	nd to 70708.6	
		Pb	mg/kg	0.001	0.01	53	126	6	nd to 12287.4	
		Sb	mg/kg	0.002	0.01				nd to 42.01	
		Se	µg/kg	1.8	9.00	0.3	109	0.2	nd to 16523.1	

		Sn	mg/kg	0.005	0.03	na	na	na	nd to 1117.66	
		Sr	mg/kg	0.001	0.01	na	na	na	nd to 10895	
		V	mg/kg	"0"	"0"	41	126	15	nd to 1064.65	
		W	mg/kg	"0"	"0"	2.3	88	2	nd to 137.39	
		Zn	mg/kg	0.006	0.028	157	126	26	0.06 to 10379.1	
Bulk Density	3B1a	Db_f	g/cm^3	na	na	na	na	na	0.15 to 2.6	0.01 g/cm^{-3}
	3B1b	Db_{33}	g/cm^3	na	na	na	na	na	0.06 to 2.53	0.01 g/cm^{-3}
	3B1c	Db_{od}	g/cm^3	na	na	na	na	na	0.07 to 2.53	0.01 g/cm^{-3}
	3B1d	Db_r	g/cm^3	na	na	na	na	na	32 to 428.8	0.01 g/cm^{-3}

*nd= not detect
*tr= trace quantity

5.5 Laboratory Sample Collection and Preparation

The purpose of any soil sample is to obtain information about a particular soil and its characteristics. Sampling provides a means to estimate the parameters of these soil characteristics with an acceptable accuracy at the lowest possible cost (Petersen and Calvin, 1986). Sub-sampling also may be used, as it permits the estimation of some characteristics of the larger sampling unit without the necessity of measurement of the entire unit. Sub-sampling reduces the cost of the investigation, but usually decreases the precision with which the soil characteristics are estimated. Efficient use of sub-sampling depends on a balance between cost and precision (Petersen and Calvin, 1986).

Soil variability and sample size are interferences to sample collection and preparation. The objective of laboratory preparation is to homogenize the soil samples used in chemical, physical, and mineralogical analyses. At each stage of sampling, an additional component of variability, the variability within the larger units, is added to the sampling error (Petersen and Calvin, 1986). Soil material needs to be adequate in amount and thoroughly mixed in order to obtain a representative sample.

Bulk Samples: Laboratory identification numbers and preparation codes are assigned to bulk soil samples. These identification numbers are unique EPA- and laboratory-assigned numbers that carry important information about the soil sample (e.g., pedon, soil horizon, location, and year sampled). Laboratory preparation codes depend on the properties of the sample and on the requested analyses. These codes carry generalized information about the characteristics of the analyzed fraction, i.e., the water content (e.g., air-dry, field-moist) and the original and final particle-size fraction (e.g., sieved <2-mm fraction processed to 75 μm) and by inference, the type of analyses performed. Identification numbers and preparation codes are reported on the Primary Characterization Data Sheets.

All soils from quarantined areas are strictly controlled under APHIS quarantine regulations 7 CFR 330.

Natural Fabrics, Clods, and Cores: Laboratory identification number and preparation codes are assigned to natural fabrics (NF), clods, and cores. These identification numbers typically relate to a corresponding bulk sample.

5.5.1 Summary of Method

For most standard chemical, physical, and mineralogical analysis, the field sample is air-dried, crushed, and sieved to <2 mm. Field-moist, fine-earth fraction samples are processed by forcing the material through a 2-mm screen by hand or with a large, rubber stopper and placed in a refrigerator for future analysis. Depending on the nature of the soil material and requested analyses, air-dry and/or field-moist samples may also be prepared as whole-soil samples or processed further to finer fractions than <2 mm. Air-dry, <75 µm sub-samples for major and trace elements are processed metal-free.

Generally, weight measurements are made and recorded on the 20- to 75-mm, 5- to 20-mm, and 2- to 5-mm fractions, with these fractions then discarded. In some cases, these weight measurements may not be recorded. Additionally, some or all of these >2-mm fractions may be processed to a finer fraction and saved for chemical, physical, and mineralogical analysis. For example, after the respective weights of the 5- to 20-mm and 2- to 5-mm fractions are recorded, these fractions may be recombined and crushed to <2 mm in a laboratory jaw crusher, with the recombined material saved for laboratory analysis. In other cases, the fine-earth fraction and the >2-mm fractions are homogenized and passed through a laboratory jaw crusher to reduce all material to pass a 2-mm sieve, with the processed material saved for laboratory analysis.

5.5.2 Interferences

Soil variability and sample size are interferences to sample collection and preparation. At each stage of sampling, an additional component of variability, the variability among smaller elements within the larger units, is added to the sampling error (Petersen and Calvin, 1986). Soil material needs to be in adequate amount and thoroughly mixed to obtain a representative sample.

Soil is mixed by moving it from the corners to the middle of processing area and then by redistributing the material. This process is repeated four times. Enough soil material needs to be sieved and weighed to obtain statistically accurate rock fragment content. In order to accurately measure rock fragments with a maximum particle diameter of 20 mm, the minimum specimen size ("dry" weight) that needs to be sieved and weighed is 1.0 kg. Refer to ASTM Standard Practice D 2488 (American Society for Testing and Materials, 2004). A homogenized soil sample is more readily obtained from air-dry material than from field-moist material. Whenever possible, "moist" samples or materials should have weights two to four times larger than for "dry" specimens (American Society for Testing and Materials, 2004). Refer to ASTM Standard Practice D 2488 (American Society for Testing and Materials, 2004).

5.5.3 Safety

Dust from sample processing is a nuisance. A mask should be worn in order to avoid breathing dust. Keep clothing and hands away from the crusher and pulverizer when these machines are in use. Use a face-shield and goggles when operating the jaw crusher. Use goggles when operating the air compressor. The HCl used to check carbonates can destroy clothing and irritate skin. Immediately rinse acid with water from clothing or skin and seek professional medical help, if needed.

5.5.4 Equipment

- Electronic Balance, ±1-g sensitivity and 15-kg capacity
- Cardboard trays for sample storage
- Trays, plastic, tared
- Sieves, square-hole, stainless steel
    - 80 mesh, 180 μm
    - 10 mesh, 2 mm
    - mesh, 4.75 mm
    - 19 mm, 3/4 in
    - 76 mm, 3 in
- 200-mesh, 75 μm
- Nylon cloth sieve
- 40-mesh, 0.425 mm
- Pulverizer
- Wooden rolling pin
- Rubber roller
- Laboratory jaw crusher, Retsch, Model BB2/A, Brinkmann Instruments Inc., Des Plaines, IL.
- Metal plate, 76 x 76 x 0.5 cm
- Containers, paper, 12-oz, with lids
- Containers, plastic, 1-pint, 1, 4, and/or 8 oz with tops
- Scintillation glass vials, 20-mL
- Metal weighing cans, 2-oz
- Brown Kraft paper
- Air compressor, Cast-iron Series, SpeedAire, Campbell Hausfeld Mfg. Co., Harrison, OH.
- Planetary ball mill, Fritsch, Model P-5, Gilson, Lewis Center, OH
- Syalon balls, 12- to 15-mm, and bowls, 80-mL
- Metal weighing cans, 2-oz
- Cross beater mill, Retsch, Brinkmann Instruments Inc., Des Plaines, IL.

5.5.5 Reagents

- Reverse osmosis (RO) water
- 1 *N* HCl
- Sodium hexametaphosphate solution. Dissolve 35.7 g of sodium hexametaphosphate $(NaPO_3)_6$ and 7.94 g of sodium carbonate (Na_2CO_3) in 1 L of RO water.

5.5.6 Procedures

1. Weigh soil sample in sample bag to nearest g when logged-in and record weight.

2. Weigh sample in sample bag to nearest g before air-drying and record weight.

3. Remove soil sample from sample bag and distribute on a plastic tray. Thoroughly mix soil material.

4. Before air-drying, weigh sample on a tared tray (tray weight) to nearest g and record weight.

5. Air-dry the sample in an oven at 30 to 35° C for 3 to 7 days.

6. Weigh sample to nearest g after air-drying and record weight.

7. Weigh sample to nearest g and record weight. This weight includes the >2-mm fractions.

8. Roll soil material on a flat, metal plate that is covered with brown Kraft paper with wooden rolling pin and/or rubber roller to crush clods to pass a 2-mm sieve. For samples with easily crushed coarse fragments, substitute a rubber roller for a wooden rolling pin. Roll and sieve until only the coarse fragments that do not slake in sodium hexametaphosphate solution remain on sieve. Crush clayey soils that contain no coarse fragments in a laboratory jaw crusher.

9. Process air-dry soil by sieving to <2-mm. Thoroughly mix material by moving the soil from the corners to the middle of the processing area and then by redistributing the material. Repeat process four times. For preparation of the >2-mm fractions, see below.

10. Procedures for air-dry, <2-mm fractions include but are not limited to the following:
    a. **Standard Analyses**: For standard chemical, physical, and mineralogical analysis, select material for representative sub-samples from at least five different areas on the plastic tray. Prepare one sub-sample of the air-dry, sieved <2-mm fraction in a 12-oz paper container.
    b. **Salt Analyses**: For a saturation paste when salt analyses are requested, select material for representative sub-samples from at least five different areas on the plastic tray. Prepare one sub-sample of the air-dry, sieved <2-mm fraction in a 1-pint plastic container.
    c. **1500-kPA**: For air-dry 1500-kPa water content of mineral and organic materials, select material for representative sub-samples from at least five different areas on the plastic tray. Prepare a sub-sample of air-dry, sieved <2-mm fraction in a 1-oz plastic cup.
    d. **Air-Dry/Oven-Dry Ratio**: For air-dry/oven-dry (ADOD) ratio (required if any air-dry analysis are determined), select material for representative sub-samples from at least five different areas on the plastic tray. Prepare a sub-sample of the air-dry, sieved <2-mm fraction in a 2-oz metal weighing can.
    e. **Presence of Carbonates**: Use a sub-sample of the ADOD sample (procedure 1B1b2b4) and check for the presence of carbonates. Reference samples (knowns) are available for comparisons. Place 1 g of the air-dry fine-earth fraction in porcelain spot plate, add reverse osmosis water, and stir to remove entrapped air. Add 1 N HCl to soil, observe amount of effervescence, and record as follows:
        i. *None* - No visual efferevescence.
        ii. *Very Slight* - Bubbles rise at a few points in the sample and consistently appear at the same point in either a steady stream of tiny bubbles or in a slower stream of larger bubbles. Do not mistake trapped air bubbles for a positive test. Generally, these air bubbles appear immediately after the addition of 1 *N* HCl.
        iii. *Slight* - More small bubbles, and possibly a few larger bubbles, appear throughout the sample than with a *very slight* reaction.
        iv. *Strong* - More large bubbles are evident than with a *slight* reaction. Often the reaction is violent at first and then quickly decreases to a reaction that produces many small bubbles.
        v. *Violent* - The sample effervesces violently. Many large bubbles appear to burst from the spot plate.
    f. **Total Carbon, Nitrogen, and Sulfur Analysis**: For total C, N, and S analyses, select material for representative sub-samples from at least five different areas on the plastic tray. Prepare one sub-sample of the air-dry, sieved <2-mm fraction

processed in a cross beater mill to (≈ 80 mesh, 180 µm) in a 20-mL scintillation glass vial.

g. **Calcium Carbonate and Gypsum**: Use the prepared sub-sample (procedure 1B1b2d1) for the determination of the amount of carbonates and/or gypsum.
h. **Chemical Analysis of Organic Materials**: For chemical analysis of organic materials, select material for representative sub-samples from at least five different areas on the plastic tray. Prepare a sub-sample of the air-dry, sieved <2-mm fraction processed in a cross beater to a fine grind (≈ 80 mesh, 180 µm) in a 12-oz paper container.
i. **Total Major and/or Trace Elements:** For total major and/or trace element analyses, select material for representative sub-samples from at least five different areas on the plastic tray. Prepare one sub-sample of the air-dry, metal free, sieved <2-mm fraction processed in a planetary ball mill for 2 min and sieved to <75 µm (200 mesh) in a 20-mL scintillation glass vial.

11. The following procedures are used for samples with >2 mm fractions. These fractions include mineral coarse fragments as well wood fragments that are >20 mm in cross section and cannot be crushed and shredded with the fingers. If the >2-mm fractions are to be weighed, recorded, and discarded, with no further laboratory analysis, proceed to procedure a. When the data for >2-mm fractions are not recorded, proceed to procedure b. If the >2-mm fractions contain carbonate- or gypsum-indurated material, and laboratory analysis is requested, proceed to procedure c. If the >2-mm fractions are Cr or R material (Soil Survey Staff, 1999), and laboratory analysis is requested, proceed to procedure d.
    a. **Particle-Size Analysis, Recorded**: Process the air-dry soil by sieving to <2 mm as described in step 9. In this procedure, weight measurements are made on the 20- to 75-mm, 5- to 20-mm, and 2- to 5-mm fractions. Weigh soil material with diameters of 2 to 5 mm. If difficult to separate the <2-mm fraction from fragments, soak (100 g of 2- to 5-mm fraction) in sodium hexametaphosphate solution for 12 h. Air-dry, weigh the material that does not slake, record weight, and discard. Weigh, record weight, and discard particles with diameters of 20 to 75 mm and 5 to 20 mm. The <2-mm fraction is saved for chemical, physical, and mineralogical analysis.
    b. **Particle-Size Analysis, Not Recorded**: This procedure is the same as described in step 9 except the weight of the >2-mm fractions are not recorded and all analytical results are reported on a <2-mm basis.
    c. **Particle-Size Analysis, Recorded**, **2- to 20-mm Fraction Processed to <2-mm, Chemical, Physical, and Mineralogical Analysis:** This procedure is commonly used for samples with carbonate- or gypsum-indurated material. Process the air-dry soil by sieving to <2 mm. Weigh soil material with diameters of 20- to 75-mm, 5 to 20-mm, and 2 to 5-mm and record weights as described in

procedure 11a. The 5- to 20-mm and 2- to 5-mm fractions are then recombined after their respective weights are recorded. The recombined, 2- to 20-mm, material is crushed to <2 mm in a laboratory jaw crusher. This material is saved for laboratory analysis and analytical results reported on the 2- to 20-mm basis. The <2-mm material is also saved for chemical, physical, and mineralogical analysis. If carbonate or gypsum accumulations are soft and easily pass a 2-mm sieve, the standard procedure is usually requested.

d. **Particle-Size Analysis, Not Recorded, Whole-Soil Processed to <2-mm, Chemical, Physical, and Mineralogical Analysis:** This procedure is mainly used to prepare samples from Cr or R soil horizons (Soil Survey Staff, 1999). Homogenize particles with diameters >2 mm and the fine-earth material (<2-mm) and crushed to <2-mm in a laboratory jaw crusher. This material is saved for laboratory analysis and analytical results reported on the whole-soil basis.

5.5.7 Calculations

Calculations for coarse fragments are reported in 3A2.

5.5.8 Report

Reported data include but are not limited to the following:

1. Weight (g) of field-moist soil sample
2. Weight (g) of air-dry soil sample
3. Weights (g) of processed air-dry soil
4. Weight (g) of 20- to 75-mm fraction
5. Weight (g) of 5- to 20-mm fraction
6. Weight (g) of 2- to 5-mm fraction
7. Weight (g) of sub-sample of 2- to 5-mm fraction before slaking
8. Weight (g) of sub-sample of 2- to 5-mm fraction after slaking
9. Effervescence with HCl (None, Very Slight, Slight, Strong, Violent)

5.5.9 Precision and Accuracy

Precision and accuracy data are available from the SSL upon request.

5.5.10 References

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5.6 Sampling Archiving

All remaining soil from a sample will be archived for potential re-analysis in the future. The remaining samples are air-dried and passed through a 2mm sieve (following procedures for sample preparation in Section 5.5.6) before storage in a climate controlled facility. Air-drying will stop most biological activity, but long-term storage may cause minor changes in concentrations of organic C, N, and S. Therefore, re-analysis of these fractions would not be appropriate.

5.7 PSDA Air-dry, <2mm Particles

5.7.1 Summary of Method

The standard SSL procedure for analysis of particles with <2-mm diameters is the air-dry method (procedure 3A1a1a), whereby a 10-g sample is pretreated to remove organic matter and soluble salts. The sample is dried in the oven to obtain the initial weight, dispersed with a sodium hexametaphosphate solution, and mechanically shaken. The sand fraction is removed from the suspension by wet sieving and then fractionated by dry sieving. The clay and fine silt fractions are determined using the suspension remaining from the wet sieving process. This suspension is diluted to 1 L in a sedimentation cylinder, stirred, and 25-mL aliquots removed with a pipet at calculated, predetermined intervals based on Stokes' law (Kilmer and Alexander, 1949). The aliquots are dried at 110°C and weighed. Coarse silt is the difference between 100% and the sum of the sand, clay, and fine silt percentages.

5.7.2 Interferences

The sedimentation equation that is used to measure the settling rates of particles of different sizes is as follows:

$$v = 2r^2 g \left(\rho_s - \rho_l\right) \left(\eta\right)$$

where:

- v = Velocity of fall
- r = Particle radius
- g = Acceleration due to gravity
- ρs = Particle density
- ρl = Liquid density
- η = Fluid viscosity

This formula results from an application of Stokes' law and is referred to as Stokes' law. Assumptions used in applying Stokes' law to soil sedimentation measurements are as follows:

1. Terminal velocity is attained as soon as settling begins.
2. Settling and resistance are entirely due to the viscosity of the fluid.
3. Particles are smooth and spherical.
4. There is no interaction between individual particles in the solution (Gee and Bauder, 1986; Gee and Or, 2002).

Since soil particles are not smooth and spherical, the radius of the particle is considered an equivalent rather than an actual radius. In this method, particle density is assumed to be 2.65 g cc^{-1}. Gypsum interferes with PSDA by causing flocculation of particles. Gypsum is removed by stirring and washing the soil with reverse osmosis water. This procedure is effective if the soil contains <25% gypsum. Partial flocculation may occur in some soils if excess H_2O_2 is not removed from the soil after its use in organic matter oxidation. Treatment of micaceous soils with H_2O_2 causes exfoliation of the mica plates and a matting of particles when dried in the oven. Since exfoliation occurs in these soils, a true measurement of fractions is uncertain (Drosdoff and Miles, 1938).

5.7.3 Precautions

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses) when handling acid and H_2O_2. Mix acids in ventilated fume hoods. Use sodium bicarbonate and water to neutralize and dilute spilled acids. Heat samples for removal of organic matter or cementing agents in ventilation hoods. Handle heated samples with leather gloves. Perform the transfer of acid to gelatin capsules near a sink in case of leakage or spills. Users should be familiar with centrifuge operation. Opposite centrifuge bottles need to be balanced. Centrifuge should not be opened until centrifuge rotor has completely stopped.

5.7.4 Equipment/Materials

- Beakers, 300 mL, tared to 1 mg
- Ceramic filter candles, 0.3 µm absolute retention
- Rack to hold ceramic filter candle and sample container.
- Mechanical shaker, horizontal, 120 oscillations min-1, 1 ½ in strokes, Eberbach 6000, Eberbach Corp., Ann Arbor, MI
- Cylinders, 1 L, white line fused onto glass at 1-L mark
- Oven, 110°C
- Hot plate, 100°C
- Vacuum, 0.8 bars (80kPa)
- Thermometer, 0 to 150°C
- Desiccator
- Motor driven stirrer, (Kilmer and Mullins, 1954)
- Hand stirrer, perforated disk fastened to a rod
- Adjustable pipet rack (Shaw, 1932; Fig.1-3)

- Lowy pipets, 25 mL, with overflow bulb
- Polyurethane foam, pipe insulation that fits snugly around cylinder.
- Sieve shaker with 12.7-mm (1/2 in) vertical and lateral movement at 500 oscillations min-1. Accommodates a nest of 76-mm (3-in) sieves.
- Weighing bottles, 90 mL, with screw caps, tared to 1 mg
- Weighing bottles, 90 mL, tared to 1 mg
- Weighing bottles, 90 mL, tared to 0.1 mg
- Drying dishes, aluminum
- Timer or clock with second hand
- Electronic balance, ±0.10-mg sensitivity
- Electronic balance, ±1.0-mg sensitivity
- Watch glass, 50- and 65-mm diameters
- Evaporating dish, porcelain, 160-mm diameter, 31-mm height, with lip
- Set of 76-mm (3 in) sieves, square weave phosphor bronze wire cloth except 300 mesh which is twilled weave. U.S. series and Tyler Screen Scale equivalent designations are as follows:

Sand Size	Opening (mm)	U.S. No.	Tyler Mesh Size
VCS	1.0	18	16
CS	0.5	35	32
MS	0.25	60	60
FS	0.105	140	150
VFS	0.047	300	300

- Centrifuge, International No. 11, with No. 949 rotor head, International Equip. Co., Boston, MA
- Centrifuge bottle, 500 mL
- Torsion balance
- Manometer, hand-held gauge and differential pressure, PCL-200A/C Series, Omega Engineering, Stamford, CT.
- Gelatin capsules, 5 mL
- Threaded weighing bottles, 90 mL
- Machined PVC caps for threaded 90-mL weighing bottles, 3.2-cm (1 1/4 in) diameter with 1.1-cm (7/16 in) diameter hole drilled in center, O-ring seal
- O-rings, 3.2 x 38.1 mm (1/8 x 1 1/2 in)
- Septa, rubber, 7.9-mm (5/16 in) diameter. Place in machined cap.
- Hypodermic needle, 25.4 mm (1 in), 23 gauge
- Ultrasonic probe, 19-mm (3/4 in) horn, 20 kHz, 300 watts

5.7.5 Reagents

- Reverse osmosis (RO) water, ASTM Type III grade of reagent water
- Hydrogen peroxide (H_2O_2), 30 to 35%
- Sodium hexametaphosphate ((NaPO3)6), reagent grade
- Sodium carbonate (Na_2CO_3), reagent grade
- Sodium hexametaphosphate solution. Dissolve 35.7 g of ($NaPO_3$)6 and 7.94 g of Na_2CO_3 in 1 L of RO water. See Section 7.12 for standardization of sodium hexametaphosphate solution.
- Ethyl alcohol
- Calcium sulfate (anhydrous) or equivalent desiccant
- Hydrochloric acid (HCl), 6 N, technical grade. Dilute 1 L of concentrated HCl with 1 L of RO water
- Sodium carbonate (Na_2CO_3) reagent. Dissolve 10.6 g Na_2CO_3 in RO water and make to 1 L (10 mg $CaCO_3$)
- 1 N sodium acetate (NaOAc) solution, buffered to pH 5. Dissolve 680 g of NaOAc in 4 L of RO water. Add ≈ 250 mL of acetic acid. Make to 5-L volume with RO water.
- Sodium citrate solution, 0.3 M $Na_3C_6H_5O_7 \cdot 2H_2O$ (88.4 g L-1)
- Sodium bicarbonate buffer solution, 1 M $NaHCO_3$ (84 g L-1)
- Sodium dithionite ($Na_2S_2O_4$ - hydrosulphite)
- Saturated NaCl solution (solubility at 20°C; 360 g L-1)
- Sodium hydroxide solution (NaOH), 0.1 N. Dissolve 4 g NaOH pellets in 1 L of RO water.

5.7.6 Procedure

1. Weigh 10g of <2-mm, air-dry soil to nearest mg on an electronic balance and place into a numbered, tared 300-mL, beaker. Wash and tare these beakers once every two months. A quality control sample is included in each batch (≤24 samples).

2. Add ≈ 50mL of RO water and 5 to 7.5 mL of H_2O_2 to the soil sample at ambient temperature. Cover the soil sample with a 50-mm watch glass. Allow initial oxidation of organic matter to complete and then place sample on hot plate. If the froth from the reaction exceeds the capacity of the beaker, transfer the sample to a larger beaker.

3. Place the sample on a hot plate and heat to 90°C. Add four 5 to 7.5-mL increments of H_2O_2 at 30-min intervals. Add additional H_2O_2 until organic matter oxidation is complete. Heat the sample for an additional 45 min to decompose excess H_2O_2. If the reaction is violent, do one or any combination of the following: (a) add small increments of ethyl alcohol to the sample; (b) remove the sample from the hot plate to slow the reaction; (c) transfer sample to

a 1000-mL beaker; or (d) reduce the amount of H_2O_2 to sample. Record any unusual sample reactions.

4. Place the sample on the filter rack. Add 150 mL of RO water. Insert a filter candle, connect to the vacuum trap assembly with tubing, and turn on the vacuum. Aspirate until liquid is removed and only slightly dampened sample remains. Wash the sample four additional times with ≈ 150mL of RO water. Stir the sample with filter candle to ensure all soil particles will be rinsed. During aspiration, it may be necessary to occasionally apply back-pressure to filter candle and remove build-up of soil which inhibits aspiration. If the sample contains gypsum and flocculates, then the following additional washings may be used. If the sample contains 1 to 5% gypsum, stir the sample with a magnetic stirrer for 5 min and wash 5 times with ≈ 250mL of RO water each time. If the sample contains >5% gypsum, place the sample in a 1000-mL beaker and stir the sample with a magnetic stirrer for 5 min then wash 5 times with ≈ 750 mL of RO water each time to remove soluble gypsum.

5. Place sample in oven. Dry the sample overnight at 110°C. Remove the sample from the oven, place in a desiccator, and cool to ambient temperature.

6. Record the total weight (TW) of the sample to the nearest mg.

7. Add the exact volume of sodium hexametaphosphate solution (≈ 10mL), equivalent to 0.4408g of sodium hexametaphosphate, to each sample. Subtract the weight of the sodium hexametaphosphate (DW) that is contained in the extracted aliquot from the silt and clay weights to calculate silt and clay percentages. To determine the exact volume of sodium hexametaphosphate to add to each sample, refer to Section 5.7.6, step 9. Let stand until sample is completely moistened by sodium hexametaphosphate. Add ≈ 175mL of RO water.

8. A sodium hexametaphosphate standardization is performed with each new batch of solution. Use only designated weighing bottles for standardization. Wash and tare these bottles after each standardization. Add duplicate aliquots (8.5, 9.0, 9.3, 9.6, 10.0, 10.3, 10.6, 11.0 mL) of sodium hexametaphosphate solution to numbered tared, 90-mL weighing bottles. Oven-dry aliquots overnight and record dry residue weight of sodium hexametaphosphate.

9. Determine the exact volume of sodium hexametaphosphate to add to each sample by regressing the volume of sodium hexametaphosphate against the dry residue weight of sodium hexametaphosphate and then by predicting the volume needed to dispense 0.4408g of sodium hexametaphosphate into each sample.

10. Place the sample in a horizontal shaker set at 120 oscillations min-1 and shake for 15 hours (overnight).

11. Remove the sample from the shaker and pour through a 300-mesh (0.047-mm) sieve mounted on a ring stand. Finger-rubbing of sample may be required during transfer to speed washing of sample. Place a funnel below the sieve and a 1-L cylinder below the funnel. Collect the silt and clay in the 1-L cylinder. Avoid using jets of water in washing the sample. Wash and rub all particles from the beaker into the sieve. Continue to wash until the suspension volume in the cylinder is ≈ 800mL. Sand and some of the coarse silt remain on the sieve. Rinse all <20-µm particles into the cylinder. Fill the cylinder to 1L and cover with a 65-mm watch glass. Place pipe insulation around sample and blank cylinders to prevent rapid changes in temperatures. Prepare a RO water blank to measure temperature fluctuations. Allow the cylinder to stand overnight to equilibrate the suspension with the room temperature. Wash the sand into an evaporation dish and dry the sand at 110°C overnight.

12. Transfer the dried sand to a nest of sieves that has a top-to-bottom order of 1.0, 0.5, 0.25, 0.1, and 0.047 mm. Shake the sand for 3 min on a shaker that has 1.3-cm vertical and lateral movements and oscillates at 500 strokes min-1. Record the weight of each separate sand fraction (SWi) to the nearest mg. If optical analysis is requested, place the very fine sand and fine sand fractions in gelatin capsules and the remaining sand fractions in a labeled vial. Store capsules in the labeled vial. Wash sand dishes after every use.

13. Determine the percentage of fine silt and clay gravimetrically by removing an aliquot from the suspension in the 1-L cylinder with a Lowy, 25-mL pipet. Periodically, gravimetrically calibrate the delivery volume of the pipet by weighing the amount of RO water dispensed from the pipet. Record the delivery volume (DV) and use the value to calculate the results. Regulate the vacuum such that the pipet fills in ≈12 s. Record temperature (T1) of blank. Mount the pipet on an adjustable pipet rack (Shaw, 1932). Stir the silt and clay suspension with mechanical stirrer for at least 5 min. Place the cylinder on a stable, vibrationless table and stir with a hand stirrer in an up-and-down motion for 30s. Timing is started upon completion of the stirring. Record the time that stirring is stopped. For the <20-µm fraction, slowly lower the closed pipet to a 10-cm depth in the suspension, turn on the vacuum, and withdraw an aliquot at the calculated time (Table 1). Dispense the aliquot into a tared and numbered, 90-mL weighing bottle. Rinse the pipet twice with RO water and dispense into the tared, weighing bottle with the aliquot. For the <2-µm fraction, pipet after a time of 4.5, 5, 5.5, or 6.5 h. Record temperature (T2) of blank. Use the average of T1 and T2 and adjust the pipet depth in the suspension as indicated in Table 2. Repeat the procedure described for the <20-µm fraction. If determination of carbonate is required, use weighing bottle with screw threads. Dry the aliquots at 110°C overnight and cool in a desiccator that

contains calcium sulfate or an equivalent desiccant. Record the weight of the residue (RW) to the nearest 0.1mg.

14. Use the 90-mL, round-bottomed, weighing bottles for the <20-µm aliquots. Wash and tare after every fourth use. Use the 90-mL, square-bottomed, weighing bottles for the <2-µm aliquots. Wash and tare after every use.

5.7.7 Reporting

Report each particle-size fraction to the nearest 0.1 percent.

5.7.8 References

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5.8 PSDA Pipet Analysis, Particles <2 mm

5.8.1 Summary of Method

Standard SSL PSDA: The standard SSL procedure for analysis of particles with <2-mm diameters is the air-dry method (procedure 3A1a1a), whereby a 10-g sample is pretreated to remove organic matter and soluble salts. The sample is dried in the oven to obtain the initial weight, dispersed with a sodium hexametaphosphate solution, and mechanically shaken. The sand fraction is removed from the suspension by wet sieving and then fractionated by dry sieving. The clay and fine silt fractions are determined using the suspension remaining from the wet sieving process. This suspension is diluted to 1L in a sedimentation cylinder, stirred, and 25-mL aliquots removed with a pipet at calculated, predetermined intervals based on Stokes' law (Kilmer and Alexander, 1949). The aliquots are dried at 110°C and weighed. Coarse silt is the difference between 100% and the sum of the sand, clay, and fine silt percentages.

Fine-Clay Determination: The soil suspension from procedure 3A1a1a is used to determine the fine-clay fraction. This suspension is stirred, poured into a centrifuge bottle, and centrifuged at 1500 rpm. A 25-mL aliquot is withdrawn with a pipet. The aliquot is dried in the oven, weighed, and the percentage of fine clay is calculated based on the total sample weight. The time of centrifugation is determined from the following equation modified from Stokes' law (Jackson, 1969).

$$t_m = (63.0x10^8 \eta \log (rs^{-1})) (N_m^2 D\mu^2 \Delta\rho)^{-1}$$

where:

t_m = Time in minutes
η = Viscosity in poises
r = Radius in cm from center of rotation to sampling depth (3 cm + s)
s = Radius in cm from center of rotation to surface of suspension
N_m = rpm (1500)
$D\mu$ = Particle diameter in microns (0.2 µm)
$\Delta\rho$ = Difference in specific gravity between solvated particles and suspension liquid
$63.0x10^8$ = Combination of conversion factors for convenient units of time in minutes, t_{min}, N_m as rpm, and particle diameter in microns, $D\mu$.

Carbonate-Clay Determination: The residue from procedure is used to determine the carbonate-clay fraction. This residue is treated with acid in a closed system. The pressure of the evolved gas is measured. The pressure is related linearly to the CO_2 content in the carbonates. A manometer is used to measure the pressure.

Pretreatment and Dispersion Techniques: In the standard PSDA, an air-dry soil sample is pretreated to remove organic matter and soluble salts. There are additional non-routine chemical pretreatments for the removal of cementing agents that often prevent complete dispersion. These pretreatments may be requested by the project coordinator as follows:

- **Carbonate Removal:** Carbonates are destroyed with a 1 N NaOAc solution buffered to pH 5. The NaOAc solution is added to sample until carbonate bubbles no longer evolve. The NaOAc solution is then washed from the sample. After destruction of carbonates, the remainder of procedure is followed.

- **Iron Removal:** Soil samples are pretreated with H_2O_2 to remove organic matter. Iron oxides are removed with bicarbonate-buffered, sodium dithionite-citrate solution and heated until the sample color changes to a grayish color. The suspension is flocculated with saturated NaCl solution and filtered to remove soluble salts. After removal of iron oxides, the remainder of procedure is followed.

- **Silica Removal:** Soils are pretreated with H_2O_2 to remove organic matter. Soils with Si cementation or coatings are pretreated with a weak NaOH solution overnight. After removal of siliceous cementing agents, the remainder of procedure is followed.

- **Ultrasonic Dispersion:** A soil sample is pretreated to remove organic matter and soluble salts. The sample is dried in the oven and weighed to obtain the initial weight. Sodium hexametaphosphate solution is added to the sample and then made

to 100-mL volume with RO water. The sample is subjected to ultrasonic vibration for 5 min. After dispersion with the ultrasonic probe, procedure is followed.

- **Water Dispersible PSDA:** Water dispersible particle-size distribution analysis may also be determined from a soil suspension without the removal of organic matter or soluble salts, or without the use of a chemical dispersant. Upon omitting these procedural steps, the remainder of procedure is followed.

5.8.2 Interferences

Standard SSL PSDA: The sedimentation equation that is used to measure the settling rates of particles of different sizes is as follows:

$$\nu = 2r^2g(\rho_s-\rho_l)/(9\eta)$$

where:

ν = Velocity of fall
r = Particle radius
g = Acceleration due to gravity
ρ_s = Particle density
ρ_l = Liquid density
η = Fluid viscosity

This formula results from an application of Stokes' law and is referred to as Stokes' law. Assumptions used in applying Stokes' law to soil sedimentation measurements are as follows:

- Terminal velocity is attained as soon as settling begins.
- Settling and resistance are entirely due to the viscosity of the fluid.
- Particles are smooth and spherical.
- There is no interaction between individual particles in the solution (Gee and Bauder, 1986; Gee and Or, 2002).
- Since soil particles are not smooth and spherical, the radius of the particle is considered an equivalent rather than an actual radius. In this method, particle density is assumed to be 2.65 g cc^{-1}.
- Gypsum interferes with PSDA by causing flocculation of particles. Gypsum is removed by stirring and washing the soil with reverse osmosis water. This procedure is effective if the soil contains <25% gypsum.
- Partial flocculation may occur in some soils if excess H_2O_2 is not removed from the soil after its use in organic matter oxidation.

- Treatment of micaceous soils with H_2O_2 causes exfoliation of the mica plates and a matting of particles when dried in the oven. Since exfoliation occurs in these soils, a true measurement of fractions is uncertain (Drosdoff and Miles, 1938).

Fine-Clay Determination: In the fine-clay determination, the distance from the center of rotation to the surface of the suspension must be constant for each centrifuge bottle. The particle density (ρp) of the fine clay is assumed to be 2.5 g cc^{-1} (Jackson, 1969). The suspension temperature must be used to enter the correct liquid viscosity in the equation. Position the bottle under pipet without sudden movement of the centrifuge rotor, which causes disturbance of solution. The withdrawal rate with pipet should be constant.

Carbonate-Clay Determination: The carbonate-clay analysis is semi-quantitative. It is assumed that all of the carbonates are converted to CO_2. This method measures all forms of carbonates. In addition to Ca, the carbonates of Mg, Na, and K also react with the acid. Analytical interferences may be caused by temperature changes within the reaction vessel. The analyst should not touch the glass of the vessel when reading the pressure. When sealing the vessel, the analyst should not hold onto the vessel any longer than necessary to tighten the cap. The internal pressure must be equalized with the atmosphere. Approximately 3 to 5 s are required to equalize the internal pressure of the bottle when piercing the septa with a needle. The analyst should replace septa and O rings at regular intervals, as they develop leaks after extensive use.

Pretreatment and Dispersion Techniques: The PSDA results are dependent on the pretreatments used to disperse the soil. The presence of cementing agents such as carbonates, Fe, and Si often prevent complete dispersion. In these cases, special pretreatment and dispersion procedures may be performed upon request on either an air-dry or field-moist sample. However, these special techniques in themselves may interfere with PSDA as follows:

- **Carbonate Removal:** The removal of carbonates with 1 *N* NaOAc (pH 5) results in sample acidification. This pretreatment can destroy the primary mineral structure of clay (Gee and Bauder, 1986).
- **Iron Removal:** If the temperature of the water bath exceeds 80° C during Fe removal, elemental S can precipitate (Mehra and Jackson, 1960). This pretreatment can destroy primary mineral grains in the clay fraction (El-Swaify, 1980).
- **Silica Removal:** The effects of Si removal with 0.1 *N* NaOH on the clay fraction and particle-size distribution are unknown.
- **Ultrasonic Dispersion5):** Ultrasonic dispersion has been reported to destroy primary soil particles. Watson (1971) summarized studies that reported the destruction of biotite and breakdown of microaggregates by ultrasonic dispersion. However, Saly (1967) reported that ultrasonic vibration did not cause the destruction of the clay crystalline

lattice or the breakdown of primary grains. The samples ranged from sandy to clayey soils. The cementing agents represented humus, carbonates, and hydroxides of Fe and Al. No standard procedures have been adopted using ultrasonic dispersion.

5.8.3 Safety

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses) when handling acid and H_2O_2. Mix acids in ventilated fume hoods. Use sodium bicarbonate and water to neutralize and dilute spilled acids. Heat samples in ventilated fume hoods for removal of organic matter or cementing agents in ventilation hoods. Handle heated samples with leather gloves. Perform the transfer of acid to gelatin capsules near a sink in case of leakage or spills.

Users should be familiar with centrifuge operation. Opposite centrifuge bottles need to be balanced. Centrifuge should not be opened until centrifuge rotor has completely stopped

5.8.4 Equipment

- Fleakers, 300 mL, tared to 1 mg
- Ceramic filter candles, 0.3 μm absolute retention (source currently unavailable)
- Rack to hold ceramic filter candle and sample container.
- Mechanical shaker, horizontal, 120 oscillations min-1, 1 ½ in strokes, Eberbach 6000, Eberbach Corp., Ann Arbor, MI
- Cylinders, 1 L, white line fused onto glass at 1-L mark
- Oven, 110°C
- Hot plate, 100°C
- Vacuum, 0.8 bars (80kPa)
- Thermometer, 0 to 150°C
- Desiccator
- Motor driven stirrer, (Kilmer and Mullins, 1954)
- Hand stirrer, perforated disk fastened to a rod
- Adjustable pipet rack (Shaw, 1932; Fig.1-3)
- Lowy pipets, 25 mL, with overflow bulb
- Polyurethane foam, pipe insulation that fits snugly around cylinder.
- Sieve shaker with 12.7-mm (1/2 in) vertical and lateral movement at 500 oscillations min-1. Accommodates a nest of 76-mm (3 in) sieves.
- Weighing bottles, 90 mL, with screw caps, tared to 1 mg

- Weighing bottles, 90 mL, tared to 1 mg
- Weighing bottles, 90 mL, tared to 0.1 mg
- Drying dishes, aluminum
- Timer or clock with second hand
- Electronic balance, ±0.10-mg sensitivity
- Electronic balance, ±1.0-mg sensitivity
- Watch glass, 50- and 65-mm diameters
- Evaporating dish, porcelain, 160-mm diameter, 31-mm height, with lip
- Set of 76-mm (3 in) sieves, square weave phosphor bronze wire cloth except 300 mesh which is twilled weave. U.S. series and Tyler Screen Scale equivalent designations are as follows:

Sand Size	Opening (mm)	U.S. No.	Tyler Mesh Size
VCS	1.0	18	16
CS	0.5	35	32
MS	0.25	60	60
FS	0.105	140	150
VFS	0.047	300	300

- Centrifuge, International No. 11, with No. 949 rotor head, International Equip. Co., Boston, MA
- Centrifuge bottle, 500 mL
- Torsion balance
- Manometer, hand-held gauge and differential pressure, PCL-200A/C Series, Omega Engineering, Stamford, CT.
- Gelatin capsules, 5 mL
- Threaded weighing bottles, 90 mL
- Machined PVC caps for threaded 90-mL weighing bottles, 3.2-cm (1 1/4 in) diameter with 1.1-cm (7/16 in) diameter hole drilled in center, O-ring seal
- O-rings, 3.2 x 38.1 mm (1/8 x 1 1/2 in)
- Septa, rubber, 7.9-mm (5/16 in) diameter. Place in machined cap.
- Hypodermic needle, 25.4 mm (1 in), 23 gauge
- Ultrasonic probe, 19-mm (3/4 in) horn, 20 kHz, 300 watts
-

5.8.5 Reagents

- Reverse osmosis (RO) water, ASTM Type III grade of reagent water
- Hydrogen peroxide (H_2O_2), 30 to 35%

- Sodium hexametaphosphate ($(NaPO_3)_6$), reagent grade
- Sodium carbonate (Na_2CO_3), reagent grade
- Sodium hexametaphosphate solution. Dissolve 35.7g of $(NaPO_3)_6$ and 7.94g of Na_2CO_3 in 1L of RO water. See Section 5.7.6, step 7 for standardization of sodium hexametaphosphate solution.
- Ethyl alcohol
- Calcium sulfate (anhydrous) or equivalent desiccant
- Hydrochloric acid (HCl), 6 N, technical grade. Dilute 1L of concentrated HCl with 1L of RO water
- Sodium carbonate (Na_2CO_3) reagent. Dissolve 10.6 g Na_2CO_3 in RO water and make to 1L (10 mg $CaCO_3$)
- 1 N sodium acetate (NaOAc) solution, buffered to pH 5. Dissolve 680 g of NaOAc in 4 L of RO water. Add ≈ 250 mL of acetic acid. Make to 5-L volume with RO water.
- Sodium citrate solution, 0.3 M $Na_3C_6H_5O_7 \cdot 2H_2O$ (88.4 g L-1)
- Sodium bicarbonate buffer solution, 1 M $NaHCO_3$ (84 g L-1)
- Sodium dithionite ($Na_2S_2O_4$ - hydrosulphite)
- Saturated NaCl solution (solubility at 20°C; 360 g L-1)
- Sodium hydroxide solution (NaOH), 0.1 N. Dissolve 4 g NaOH pellets in 1 L of RO water.

5.8.6 Procedures

1. Weigh 10g of <2-mm, air-dry soil to nearest mg on an electronic balance and place into a numbered, tared 300-mL, fleaker. Wash and tare these fleakers once every two months. A quality control sample is included in each batch (<24 samples).

2. Add ≈ 50mL of RO water and 5 to 7.5mL of H_2O_2 to the soil sample at ambient temperature. Cover the soil sample with a 50-mm watch glass. Allow initial oxidation of organic matter to complete and then place sample on hot plate. If the froth from the reaction exceeds the capacity of the fleaker, transfer the sample to a larger beaker.

3. Place the sample on a hot plate and heat to 90°C. Add four 5 to 7.5-mL increments of H_2O_2 at 30-min intervals. If oxidation is incomplete, add additional H_2O_2 until organic matter oxidation is complete. Heat the sample for an additional 45 min to decompose excess H_2O_2. If the reaction is violent, do one or any combination of the following: (a) add small increments of ethyl alcohol to the sample; (b) remove the sample from the hot plate to slow the reaction; (c) transfer sample to a 1000-mL beaker; or (d) reduce the amount of H_2O_2 to sample. Record any unusual sample reactions.

4. Place the sample on the filter rack. Add 150mL of RO water. Insert a filter candle, connect to the vacuum trap assembly with tubing, and turn on the vacuum. Aspirate until liquid is removed and only slightly dampened sample remains. Wash the sample four additional times with ≈ 150mL of RO water. Stir the sample with filter candle to ensure all soil particles will be rinsed. During aspiration, it may be necessary to occasionally apply back-pressure to filter candle and remove build-up of soil which inhibits aspiration. If the sample contains gypsum and flocculates, then the following additional washings may be used. If the sample contains 1 to 5% gypsum, stir the sample with a magnetic stirrer for 5 minutes and wash 5 times with ≈ 250mL of RO water each time. If the sample contains >5% gypsum, place the sample in a 1000-mL beaker and stir the sample with a magnetic stirrer for 5 minutes then wash 5 times with ≈ 750mL of RO water each time to remove soluble gypsum.

5. Place sample in oven. Dry the sample overnight at 110°C. Remove the sample from the oven, place in a desiccator, and cool to ambient temperature.

6. Record the total weight (TW) of the sample to the nearest mg.

7. Add the exact volume of sodium hexametaphosphate solution (≈ 10 mL), equivalent to 0.4408 g of sodium hexametaphosphate, to each sample. Subtract the weight of the sodium hexametaphosphate (DW) that is contained in the extracted aliquot from the silt and clay weights to calculate silt and clay percentages. To determine the exact volume of sodium hexametaphosphate to add to each sample, refer to Section 5.7.6, step 9. Let stand until sample is completely moistened by sodium hexametaphosphate. Add ≈ 175 mL of RO water.

8. A sodium hexametaphosphate standardization is performed with each new batch of solution. Use only designated weighing bottles for standardization. Wash and tare these bottles after each standardization. Add duplicate aliquots (8.5, 9.0, 9.3, 9.6, 10.0, 10.3, 10.6, 11.0 mL) of sodium hexametaphosphate solution to numbered tared, 90-mL weighing bottles. Oven-dry aliquots overnight and record dry residue weight of sodium hexametaphosphate. Determine the exact volume of sodium hexametaphosphate to add to each sample by regressing the volume of sodium hexametaphosphate against the dry residue weight of sodium hexametaphosphate and then by predicting the volume needed to dispense 0.4408g of sodium hexametaphosphate into each sample.

9. Place the sample in a horizontal shaker set at 120 oscillations min^{-1} and shake for 15 hours (overnight).

10. Remove the sample from the shaker and pour through a 300-mesh (0.047-mm) sieve mounted on a ring stand. Finger-rubbing of sample may be required during transfer to

speed washing of sample. Place a funnel below the sieve and a 1-L cylinder below the funnel. Collect the silt and clay in the 1-L cylinder. Avoid using jets of water in washing the sample. Wash and rub all particles from the fleaker into the sieve. Continue to wash until the suspension volume in the cylinder is ≈ 800mL. Sand and some of the coarse silt remain on the sieve. Rinse all <20-μm particles into the cylinder. Fill the cylinder to 1L and cover with a 65-mm watch glass. Place pipe insulation around sample and blank cylinders to prevent rapid changes in temperatures. Prepare a RO water blank to measure temperature fluctuations. Allow the cylinder to stand overnight to equilibrate the suspension with the room temperature. Wash the sand into an evaporation dish and dry the sand at 110°C overnight.

11. Transfer the dried sand to a nest of sieves that has a top-to-bottom order of 1.0, 0.5, 0.25, 0.1, and 0.047 mm. Shake the sand for 3 minutes on a shaker that has 1.3-cm vertical and lateral movements and oscillates at 500 strokes min-1. Record the weight of each separate sand fraction (SWi) to the nearest mg. If optical analysis is requested, place the very fine sand and fine sand fractions in gelatin capsules and the remaining sand fractions in a labeled vial. Store capsules in the labeled vial. Wash sand dishes after every use.

12. Determine the percentage of fine silt and clay gravimetrically by removing an aliquot from the suspension in the 1-L cylinder with a Lowy, 25-mL pipet. Periodically, gravimetrically calibrate the delivery volume of the pipet by weighing the amount of RO water dispensed from the pipet. Record the delivery volume (DV) and use the value to calculate the results. Regulate the vacuum such that the pipet fills in ≈12 seconds. Record temperature (T1) of blank. Mount the pipet on an adjustable pipet rack (Shaw, 1932). Stir the silt and clay suspension with mechanical stirrer for at least 5 minutes. Place the cylinder on a stable, vibrationless table and stir with a hand stirrer in an up-and-down motion for 30 seconds. Timing is started upon completion of the stirring. Record the time that stirring is stopped. For the <20-μm fraction, slowly lower the closed pipet to a 10-cm depth in the suspension, turn on the vacuum, and withdraw an aliquot at the calculated time (Table 1). Dispense the aliquot into a tared and numbered, 90-mL weighing bottle. Rinse the pipet twice with RO water and dispense into the tared, weighing bottle with the aliquot. For the <2-μm fraction, pipet after a time of 4.5, 5, 5.5, or 6.5 hours. Record temperature (T2) of blank. Use the average of T1 and T2 and adjust the pipet depth in the suspension as indicated in Table 2. Repeat the procedure described for the <20-μm fraction. If determination of carbonate is required, use weighing bottle with screw threads. Dry the aliquots at 110°C overnight and cool in a desiccator that contains calcium sulfate or an equivalent desiccant. Record the weight of the residue (RW) to the nearest 0.1mg.

13. Use the 90-mL, round-bottomed, weighing bottles for the <20-μm aliquots.

14. Wash and tare after every fourth use. Use the 90-mL, square-bottomed, weighing bottles for the <2-μm aliquots. Wash and tare after every use.

15. If optical mineralogy, fine-clay and/or carbonate clay determinations are not requested, the procedural aspects of the standard air-dry PSDA for the <2-mm fraction are complete. If optical mineralogy is requested, save the sediment and proceed on with the section on optical mineralogy. If fine-clay and/or carbonate clay are requested, proceed on with the sections on these analyses.

Optical Mineralogy

16. If optical mineralogy is requested, decant the suspension and transfer the sediment to a 400-mL beaker. Fill the beaker to a 5.5-cm height. Stir the sediment and allow it to settle for 5 minutes. Discard the supernatant. Refill the beaker to 5.5-cm height. Stir again, allow it to settle for 3 minutes, and then decant. Repeat the filling and the stirring; allow it to settle for 2 minutes; decant until top half of suspension is clear. Transfer the sediment, which is dominantly 20 to 50μm, to a labeled drying dish. Wash with ethanol, air-dry, and save in the drying dish for optical mineralogy.

Fine Clay Determination (<0.2 μm)

17. Stir the silt and clay suspension with mechanical stirrer for 5 minutes. Remove sample from mechanical stirrer and place on table. Stir with the hand stirrer in an up-and-down motion for 30 seconds and allow the suspension to settle for 15 minutes.

18. Pour the suspension into a centrifuge bottle and fill to the line marked on the bottle. The marked line on each bottle is 13cm which is the distance from the center of rotation to the surface of the suspension. Stopper and shake well to mix the suspension.

19. Balance opposite centrifuge loads, which consist of centrifuge bottle, trunnion carrier and bucket. Place loads on a torsion balance and add water to the lighter bucket until both loads weigh the same.

20. Read the temperature of the suspension.

21. Centrifuge at 1500 rpm.

22. Vary the centrifuge time according to the temperature as follows:

Temperature (°C)	Viscosity η	Delta-Density $\Delta\rho$	Time (Minutes)
18	0.01055	1.501	39.0
19	0.01029	1.501	38.0
20	0.01004	1.502	37.1
21	0.00980	1.502	36.2
22	0.00957	1.502	35.3
23	0.00934	1.502	34.5
24	0.00913	1.502	33.7
25	0.00892	1.503	32.9
26	0.00872	1.503	32.2
27	0.00853	1.503	31.4
28	0.00834	1.503	30.8
29	0.00816	1.504	30.1
30	0.00799	1.504	29.4

where:

s = 15 cm
r = 18 cm
Nm = 1500 rpm
ρp = 2.5 g cc-1

23. After centrifuging, lower the pipet to a 3-cm depth in the suspension. Withdraw a 25-mL aliquot at a rate of ≈ 12 seconds. Avoid turbulence. Transfer the aliquot to a weighing bottle.

24. Place weighing bottle with aliquot in oven. Dry overnight at 110°C. Remove sample from oven, place in desiccator with calcium sulfate or equivalent desiccant, and cool to ambient temperature.

25. Weigh residue weight (RW) to nearest 0.1 mg.

26. Use the 90-mL, round-bottomed, weighing bottles for the <0.2µm aliquots. Wash and tare after every fourth use.

Carbonate Clay (<2 µm): Manometer Calibration

27. Calibrate the manometer quarterly or whenever equipment changes. Calibrate by placing replicated aliquots of 0.0 to 20.0 mL of the Na_2CO_3 reagent into numbered, tared, 90-mL weighing bottles. Dry the standard samples in the oven overnight at 110°C. Remove

samples from oven, place in desiccator and cool to ambient temperature. Record the weight of the standard samples to nearest 0.1mg.

28. Lubricate the lip of the 90-mL, weighing bottle that contains the Na_2CO_3 with a thin film of glycerine. Dispense 3 mL of 6 N HCl into a gelatin capsule and place the top on the capsule. If HCl leaks from the capsule, discard the capsule. Place the capsule into the glass bottle and immediately cap the bottle. Release pressure in the bottle by piercing the septa with a hypodermic needle which is not connected to the manometer. Allow 3 to 5 seconds for internal pressure in bottle to equalize.

29. After the gelatin capsule has dissolved (several minutes), slowly tip the bottle and rotate it to saturate the standard sample adhering to the sides of the bottle. Avoid changing the temperature of the container by only handling the cap. Allow sample to stand for at least 30 minutes.

30. Adjust the manometer to zero before taking measurements. Insert the hypodermic needle in the septa stopper which is connected to the transducer. Measure the pressure inside the weighing bottle. Record the manometer readings (mm Hg) to the nearest whole number.

31. Calculate the linear regression equation, i.e., the dependent variable is the Na_2CO_3 weights (regressed or predicted values) and the independent variable is the corresponding manometer readings.

Carbonate Clay: Analysis

32. Determine the presence of carbonates in <2-mm soil by placing soil on a spot plate and adding two or three drops of 1 N HCl. The rate of CO_2 evolution indicates the relative amount of carbonates (1B1b2b5).

33. If the soil contains more than a “slight” amount of carbonates, determine the amount of carbonate clay in the <2-µm dry residue. Use the 90-mL, square-bottomed, weighing bottles for the <2-µm aliquots and carbonate determination. Wash and tare after every use.

34. With a thin film of glycerine, lubricate the lip of the 90-mL, weighing bottle that contains the <2-µm residue. In each analysis batch, include an empty weighing bottle as a blank. Dispense 3 mL of 6 N HCl into a gelatin capsule and place the top on the capsule. If HCl leaks from the capsule, discard the capsule. Place the capsule into the glass bottle and immediately cap the bottle. Release any pressure in the bottle by piercing the septa with a hypodermic needle that is not connected to the manometer. Approximately 3 to 5 seconds are required to equalize the internal pressure of the bottle.

35. After the gelatin capsule has dissolved (several minutes), slowly tip the bottle and rotate it to saturate the clay adhering to the sides of the bottle. Handle only the cap to avoid changing the temperature of the container. Allow sample to stand for at least 30 minutes.

36. Adjust the manometer to zero before taking measurements. Insert the hypodermic needle in septa stopper which is connected to the transducer. Measure the pressure inside the weighing bottle and record the manometer readings (MR) to the nearest whole number (mm Hg). Begin readings with the blank (BR).

37. Compare the sample readings with those of a standard curve prepared by measuring CO_2 evolved from a series of Na_2CO_3 aliquots with a range 0 to 200 mg.

NOTE: Additional non-routine PSA method instructions described in SSIR-42.

5.8.7 Calculations

Use these calculations for all PSDA procedures. Calculations 1 – 6 are as follows:

1. Clay % = 100 x ((RW 2 - DW) x (CF/TW))

   where:
   - RW_2 = Residue weight (g), <2-μm fraction
   - DW = Dispersing agent weight (g) = (0.4408/CF)
   - CF = 1000 mL/DV
   - DV = Dispensed pipet volume
   - TW = Total weight (g), H_2O_2-treated, oven-dry sample

2. Fine Silt % = 100 x ((RW20-DW) x (CF/TW)) - Clay %

   where:
   - RW_{20} = Residue weight (g) of <20-μm fraction

3. Sand % = $\sum$ (SWi /TW) x 100

   where:
   - SW_i = Weight of sand fractions (1.0, 0.5, 0.25, 0.1, and 0.047 mm)

4. Coarse silt % = 100 - (Clay % + Fine Silt % + Sand %)

5. Fine Clay (%) = 100 x ((RW-DW) x (CF/TW))

   where:
   - RW = Residue weight (g) of <0.2-µm fraction
   - DW = Dispersing agent weight (g) = (0.4364/CF)
   - CF = 1000 mL/DV
   - DV = Dispensed pipet volume
   - TW = Total weight of H_2O_2-treated, oven-dry sample

6. Calculate carbonate clay percentage as follows:

   Correct the manometer reading as follows:

   CR = (MR - BR)

   where:
   - CR = Corrected reading
   - MR = Manometer reading
   - BR = Blank reading

   Three blanks are run with each batch (≤24 samples). The average of three blanks is used as BR.

   Calculate two regression equations, i.e., one for corrected manometer readings <100 and another for corrected readings ≥100. Use the Na_2CO_3 weights as the dependent variable (regressed or predicted values) and the corresponding manometer readings as the independent variable.

   Use the corrected (CR) linear regression equations to estimate the g of $CaCO_3$ in the sample.

   Carbonate Clay Equivalent (<2 µm) (%) = ((g $CaCO_3$) x 100 x CF)/TW

   where:
   - CF = 1000 mL/dispensed pipet volume (mL)
   - TW = Total weight of H_2O_2-treated oven-dry sample

   Noncarbonate Clay (<2 µm) (%) = Total Clay (%) - Carbonate Clay Equivalent (%)

5.8.8 Report

Report each particle-size fraction to the nearest 0.1 percent.

5.8.9 Precision and Accuracy

Precision and accuracy data are available from the SSL upon request.

5.8.10 References

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5.9 Water Retention, 1500-kPa

5.9.1 Summary of Method

The pressure desorption procedure (U.S. Salinity Laboratory Staff, 1954) is used. A sample of <2-mm (sieved), air-dry soil is placed in a retainer ring sitting on a cellulose membrane in a pressure-membrane extractor. The membrane is covered with water to wet the samples by capillarity. The sample is equilibrated at 1500 kPa. The pressure is kept constant until equilibrium is obtained (Klute, 1986). The gravimetric water content is determined.

5.9.2 Interferences

A leaking pressure extractor prevents equilibration of samples. Check outflow air to verify that the pressure membrane extractor is functioning properly and does not leak. The pressure should be monitored for stability. Equilibration must be done at constant temperature and humidity. Samples that do not wet by capillarity are moistened with ethyl alcohol.

Laboratory-determined, water retention data are usually higher than field-determined, water retention data because the confining soil pressure is not present in the laboratory (Bruce and Luxmoore, 1986).

Aerated 0.005 *M* $CaSO_4$ has also been recommended (Dane and Hopmans, 2002), especially for fine-textured soils that contain significant amounts of swelling clays. Distilled or deionized water can possibly promote dispersion of clays in samples and freshly drawn tap water is often supersaturated with air, affecting the water content at a given pressure head (Dane and Hopmans, 2002).

5.9.3 Safety

High-pressure plumbing must be maintained in good working order. Ensure that the pressure is zero before removing bolts from the pressure-apparatus lid. Ensure that the bolts are tightened before applying pressure. Do not drop the heavy lid.

5.9.4 Equipment

- Pressure membrane extractor (Fig.1 and 2)
- Cellulose membrane
- Retainer rings. Use 10-mm height and 50-mm diameter rings for organic soils and 10-mm height and 40-mm diameter rings for all other soils.
- Electronic balance, ±0.01-g sensitivity
- Oven, 110°C
- Pressure source, regulator, and gauge
- Metal weighing cans, tared, with lids
- Vacuum trap assembly
- Vacuum, 80 kPa (0.8 bar)

5.9.5 Reagents

- Ethyl alcohol, 95%, technical grade
- Reverse osmosis (RO) water

5.9.6 Procedure

1. Submerge a cellulose membrane in RO water for 12 h or more before use. Install the wet cellulose membrane in the pressure extractor.

2. Add water and retaining rings. Add enough water to keep membrane moist. Water level should be less than height of retaining rings. Use 5-cm diameter rings for soils that are >12% in organic matter. Use 4-cm diameter rings for all other soils.

3. Fill retaining rings with 10 to 15 g of <2-mm or fine-grind, air-dry soil sample. Include a quality control (QC) sample with each plate. Continue to add water until all samples have moistened by capillarity. If samples do not moisten, apply ethyl alcohol to the surface of the

sample. Allow ethyl alcohol to evaporate. Cover samples with a sheet of plastic to reduce evaporation, close the extractor, and let stand overnight.

4. Remove excess water on the plate with a vacuum and trap assembly.

5. Assemble the extractor and uniformly tighten the bolts. Torque the bolts on both sides of the hinge to 138.0 kPa (200 psi). Torque the remaining bolts to 103.5 kPa (150 psi).

6. Increase air pressure ≈ 150 kPa every 15 min until 1500 kPa is reached. After 4 h, apply the pressure differential by closing the valve that joins the mercury circuit and by opening the pressure release valve until air is forced through the mercury. Quickly close the pressure release valve. This forces the rubber diaphragm against the top of the samples. The samples are equilibrated when water ceases to emit from the outflow tube.

7. At equilibrium, open the extractor and quickly transfer the samples to water cans, cover with lids, and record the weights (Ms+w).

8. Remove the lids, place samples in the oven, and dry at 110°C overnight. Remove samples from the oven, replace the lids, allow cans to cool to ambient temperature, and record the weights (Ms).

9. Record the weights of the empty cans (Mc).

5.9.7 Calculations

$$H_2O\ \% = 100 \times [(M_{s+w} - M_s)/ (M_s - M_c)]$$

where:

H_2O % = Percent gravimetric water content
M_{s+w} = Weight of solids + H_2O + container
M_s = Weight of solids + can
M_c = Weight of container

Gypsiferous soils are a special case because gypsum ($CaSO_4 \cdot 2H_2O$) loses most of its two water molecules at 105°C. Properties of gypsiferous soils such as 1500-kPa water content that are reported on an oven-dry weight basis are converted to include the weight of crystal water in gypsum. Refer to procedure 3D3 for these conversion calculations. The 1500-kPa water content is corrected when the gypsum content of the soil is >1%. Gypsum content of the soil is determined in procedure 4E2a1a1.

5.9.8 Report

Report water content to the nearest 0.1 percent.

5.9.9 Precision and Accuracy

Precision and accuracy data are available from the SSL upon request.

5.9.10 References

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5.10 Calcium Carbonate Equivalent

5.10.1 Summary of Method

The amount of carbonate in the soil is measured by treating the samples with HCl. The evolved CO_2 is measured manometrically. The amount of carbonate is then calculated as percent $CaCO_3$.

5.10.2 Interferences

Chemical interference is the reaction by the acid with other carbonates, e.g., carbonates of Mg, Na, and K, that may be present in soil sample. The calculated $CaCO_3$ is only a semiquantitative measurement (Nelson, 1982). Analytical interference may be caused by temperature changes within the reaction vessel. When sealing the vessel, the analyst should not hold the vessel any longer than necessary to tighten the cap. The internal pressure must be equalized with the atmosphere. After the septum has been pierced with a needle, ≈ 5 to 10 s are required to equalize the internal pressure of the bottle. With extensive use, the septa leak gas under pressure. The septa should be replaced at regular intervals. The analyst should not touch the glass of the vessel when reading the pressure.

5.10.3 Precautions

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses) when handling acids. Thoroughly wash hands after handling acids. Use the fume hood when diluting concentrated HCl. Use the safety showers and eyewash stations to dilute spilled acids. Use sodium bicarbonate and water to neutralize and dilute spilled acids.

The gelatin capsule may leak acid while being filled. Keep other personnel away from the area when filling capsules.

High pressure may develop inside the bottle if there is a large amount of calcareous sample. Do not use more than 2 g of any sample in the bottles. If high pressure develops in the bottle, release the pressure by venting the gas with a syringe needle. Some bottles may break without shattering. Discard any bottle with hairline cracks or obvious defects.

5.10.4 Equipment/Materials

- Electronic balance, ±0.10-mg sensitivity
- Electronic balance, ±1mg-sensitivity
- Threaded weighing bottles, wide-mouth, clear glass, standard, 120 mL (4 fl. oz.), 48-mm neck size. For best results, grind rim of bottle with 400-600 grit sandpaper on a flat glass plate.
- Machined PVC caps for threaded 120-mL (4 fl. oz.) weighing bottles, 54-mm diameter with 12.7-mm diameter hole drilled in center, O-ring seal.
- O-rings, 3.2 x 50.8 x 57.2 mm (1/8 x 2 x 2 1/4 in)
- Flanged stopper No. 03-255-5, Fisher Scientific. Place in machined cap.
- Manometer, hand-held gauge and differential pressure, PCL-200 Series, Omega Engineering, Stanford, CT.
- Hypodermic needle, 25.4 mm (1 in), 23 gauge. Connect needle to pressure tubing on transducer.
- Mechanical rotating shaker, 140 rpm, Eberbach 6140, Eberbach Corp., Ann Arbor, MI.

5.10.5 Reagents

- Reverse osmosis deionized (RODI) water, ASTM Type I grade of reagent water
- Methyl red indicator
- Hydrochloric acid (HCl), concentrated, 12 N
- HCl, 3 N. Dilute 500 mL of concentrated HCl with 1500 mL RODI water. Add a few crystals of methyl red indicator. Methyl red indicator will turn yellow if HCl is consumed by sample. If this reaction occurs, adjust the sample size (smaller).
- Gelatin capsule, 10 mL, size 11, Torpac Inc., Fairfield, NJ.
- Glycerin, USP. Put the glycerin in a small squeeze bottle and use as a lubricant for the O-rings.
- $CaCO_3$, Ultrex, assay dried basis 100.01%.

5.10.6 Procedure

Manometer Calibration: Calibrate the manometer quarterly or whenever equipment changes (e.g., old rubber septum replaced). Calibrate by weighing three replicates of $CaCO_3$ standards (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75 g). Weigh to the nearest 0.1mg. Dry the standard samples in the oven for 2 h at 110°C. Remove samples from oven, place in desiccator and cool to ambient temperature.

<2-mm Basis: A $CaCl_2$ pH >6.95 is generally used as an indicator of the presence of carbonates. The presence of carbonates (effervescence with HCl) is also checked during lab preparation.

1. Weigh 0.5 to 2 g of fine-grind, air-dry soil sample to the nearest mg and place in a 120-mL, wide-mouth bottle. Run 3 blanks and a quality control check sample with every batch of 24 samples. The quality control check sample serves as a single point check. Vary the sample weight according to the $CaCO_3$ content based on effervescence of sample as follows:

2. Use a 2-g sample weight if effervescence is None, Very Slight, or Slight.

3. Use a 1-g sample weight, if effervescence is Strong.

4. Use a 0.5-g sample weight, if effervescence is Violent.

5. Lubricate the O-ring of bottle cap with glycerin from a squeeze bottle.

6. Dispense 10 mL of 3 N HCl into a gelatin capsule and carefully place the top on the capsule. The HCl may squirt or leak out of capsule. If this happens, discard the capsule.

7. Place the capsule in bottle and cap bottle immediately.

8. Release any pressure in the bottle by piercing the stopper with a hypodermic needle. Remove the needle after ≈ 5 to 10 s.

9. After 5 to 10 min, the HCl dissolves through the capsule. Shake the bottle at a rate of 140 rpm on the shaker for the first 10 min and last 10 min of a 1-h interval at room temperature (20°C ± 2°C). After this 1 h, measure the pressure in the bottle by piercing the stopper of the cap with a hypodermic needle connected to the manometer.

10. Auto-zero the manometer before taking readings. Record the manometer readings (mm Hg).

5.10.7 Calculation

Correct the manometer readings as follows:

$$CR = (MR – BR)$$

where:

CR = Corrected reading
MR = Manometer reading

BR = Blank reading

Three blanks are run with each batch of 24 samples. The average of three blanks is used as BR.

Calculate the regression equation for the corrected manometer readings. Use the $CaCO_3$ weights as the dependent variable (regressed or predicted values) and the corresponding manometer readings as the independent variable.

Use the corrected (CR) linear regression (slope, intercept) equation to estimate % $CaCO_3$ in the sample as follows:

CCE = [(CR x Slope + Intercept) /Sample Weight (g)] x AD/OD

where:

CCE = Calcium Carbonate Equivalent (%) in <2-mm fraction or 2- to 20-mm fraction
CR = Corrected manometer reading
AD/OD = Air-dry/oven-dry ratio (procedure 3D1)

Carbonate = (A x B) + [C x (1-B)]

where:

Carbonate = Carbonate as $CaCO_3$ on a <20-mm basis (%)
A = $CaCO_3$ in <2-mm fraction (%)
B = Weight of the <20-mm fraction minus the weight of the 2- to 20-mm fraction divided by the weight of <20-mm fraction (procedures 1B2b2f and 3A2).
C = $CaCO_3$ in 2- to 20-mm fraction (%)

5.10.8 Reporting

Report $CaCO_3$ equivalent as a percentage of oven-dry soil to the nearest whole number.

5.10.9 References

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5.11 Total Carbon, Total Nitrogen, and Total Sulfur

5.11.1 Summary of Method

An air-dry (80 mesh, <180 µm) sample is packed in a tin foil, weighed, and analyzed for total C, N, and S by an elemental analyzer. The elemental analyzer works according to the principle of catalytic tube combustion in an oxygenated CO_2 atmosphere and high temperature. The combustion gases are freed from foreign gases. The desired measuring components (N_2, CO_2, and SO_2) are separated from each other with the help of specific adsorption columns and determined in succession with a thermal conductivity detector, with helium as the flushing and carrier gas.

5.11.2 Interferences

Contamination through body grease or perspiration must be avoided in sample packing. Substance loss after weighing should be avoided by exact folding of the sample into the tin foil. Air in the sample material should be minimized (falsifying the N value) by compressing the sample packing. Insufficient O_2 dosing reduces the catalysts, decreasing their effectiveness and durability. Burnt sample substance that remains in ash finger falsifies the results of subsequent samples. WO_3 is used as sample additive and combustion filling to aid combustion or bind interfering substances (alkaline or earth-alkaline elements, avoid non-volatile sulfates).

5.11.3 Precautions

Exhaust gas pipes should lead into a ventilated fume hood. Aggressive combustible products should not be analyzed. Before working on electrical connections (adsorption columns) or before changing reaction tubes, the instrument must be cooled down and cooled off. Gloves and safety glasses should be worn at all times during operation and maintenance of instrument.

5.11.4 Equipment/Materials

Elemental analyzer with on-line electronic balance (0.1 mg sensitivity) and automatic sample feeder, Elementar varioEL and Elementar varioEL III, Elementar Analysensysteme GmbH, Hanau-Germany, and combustibles (Elementar Americas, Inc., Mt. Laurel, NJ; Alpha Resources Inc., Stevensville, MI) as follows:

- Quartz ash finger, quartz
- Quartz bridge
- Combustion tube
- Reduction tube
- Gas purification (U-tube, GL 18)
- Support tube (65 mm)
- Protective tube
- O2 lance (150 mm rapid N)
- Tin boats (4 x 4 x 11 mm)
- Tin foil cups
- Computer, with varioEL software, Elementar Analysensysteme GmbH, Hanau-Germany, and printer.

5.11.5 Reagents

- Sulfanilic acid, calibration standard, 41.6% C, 4.1% H, 8.1 % N, 27.7% O, and 18.5% S
- Copper sticks
- Corundum balls, high purity, alumina spheres, 3 – 5 mm
- Cerium dioxide, 1 – 2 mm
- Tungsten oxide powder, sample additive
- Tungsten trioxide granulate, combustion tube filling
- Quartz wool
- Silver wool
- Phosphorus pentoxide, Sicapent, Elementar Americas, Inc., Mt. Laurel, NJ
- Helium, carrier gas, 99.996% purity
- Oxygen, combustion gas, 99.995% purity

5.11.6 Procedure

1. Elemental Analyzer Set-up and Operation – Refer to the manufacturer's manual for operation and maintenance of the elemental analyzer. Conditioning of the elemental analyzer and determination of factor and blank value limit are part of the daily measuring routine. The following are only very general guidelines for instrument parameters for the various analytes in the CNS mode.
    a. Temperature

Furnace 1	1140° C
Furnace 2	850 ° C
Furnace 3	0 ° C
CO_2 Column	85 ° C
SO_2 Column	210 ° C
SO_2 Col. Standby	140 ° C

    b. Timing

Flush	5 s
Oxygen Delay	10 s
Auto Zero Delay	30 s
Integrator Reset Delay	50 s
Peak Anticipation N	70 s
Peak Anticipation C	125 s
Peak Anticipation S	70 s

    c. Integrated Reset Delay for S 60 s
    d. Thresholds

N Peak	3 mV

C Peak	3 mV
S Peak	3 mV

e. O2 Dosing

Index 1	150 s
Index 2	1 s
Index 3	90 s
Index 4	120 s
Index 5	180 s

2. Elemental Analyzer Calibration and Analysis
   a. A calibration that covers the desired working range of each element is performed. The test substances sulfanilic acid with each given element content and different weights is analyzed. The PC program automatically computes the calibration function (linear, polynomial, or mixed). Calibration will typically remain stable for at least 6 months. Re-calibration is recommended when the daily factor is outside the range of 0.9 to 1.1 or if components that influence the results (e.g., detector or adsorption column) have been exchanged. Changing the desorption temperature of adsorption columns can also require a re-calibration.
   b. Add 0.100 g of tungsten oxide in tin foil and tare. An homogenized fine-grind, air-dry soil sample is then packed in this tin foil, weighed (0.100 to 0.05 g), and placed into the carousel of the automatic sample feeder of elemental analyzer. Sample weight is based on visual observation of the sample, related to element content, homogeneity, and combustion behavior of the sample. The sample weight is entered in the PC from an on-line electronic balance via an interface. A quality control (QC) sample is performed at a minimum of every 35 to 40 samples.

5.11.7 Calculation

$$C\ (\%) = C_i \times AD/OD$$

where:

C (%) = C (%), oven-dry basis
Ci = C (%) instrument
AD/OD = Air dry/oven-dry ratio

$$N\ (\%) = N_i \times AD/OD$$

where:

N (%) = N (%), oven-dry basis
N_i = N (%) instrument
AD/OD = Air-dry/oven-dry ratio

$$S\ (\%) = S_i \times AD/OD$$

where:

S (%) = S (%) on oven-dry basis
S_i = S (%) instrument
AD/OD = Air-dry/oven-dry ratio

5.11.8 Reporting

Report total C and S percentages to the nearest 0.01% and total N to the nearest 0.001%.

5.11.9 References

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5.12 pH

5.12.1 Summary of Method

The pH is measured in soil-water (1:1) and soil-salt (1:2 $CaCl_2$) solutions. For convenience, the pH is initially measured in water and then measured in $CaCl_2$. With the addition of an equal volume of 0.02 M $CaCl_2$ to the soil suspension that was prepared for the water pH, the final soil-solution ratio is 1:2 0.01 M $CaCl_2$.

A 20-g soil sample is mixed with 20 mL of reverse osmosis (RO) water (1:1 w:v) with occasional stirring. The sample is allowed to stand 1 h with occasional stirring. The sample is stirred for 30 s, and the 1:1 water pH is measured. The 0.02 *M* $CaCl_2$ (20mL) is added to soil suspension, the sample is stirred, and the 1:2 0.01 *M* $CaCl_2$ pH is measured (4C1a2a2).

5.12.2 Interferences

The pH will vary between the supernatant and soil sediment (McLean, 1982). Measure the pH just above the soil sediment to maintain uniformity. Clays may clog the KCl junction and slow the electrode response. Clean the electrode. Wiping the electrode dry with cloth, laboratory tissue or similar material may cause electrode polarization. Rinse the electrode with distilled water and pat dry.

Atmospheric CO_2 affects the pH of the soil:water mixture. Closed containers and nonporous materials will not allow equilibration with CO_2. At the time of pH determination, the partial pressure of CO_2 and the equilibrium point must be considered, if doing critical work.

5.12.3 Precautions

No significant hazards are associated with the procedure. Follow standard laboratory safety practices.

5.12.4 Equipment/Materials

- Measuring scoop, handmade, ≈ 20-g capability
- Paper cup, 120 mL (4 fl. oz.), disposable, Solo Cup Co., No. 404
- Dispenser, 0 to 30 mL, Repipet or equivalent
- Beverage stirring sticks, wood
- Titration beakers, polyethylene, 250 mL
- Automatic titrator, Metrohm Titroprocessors, Control Units, Sample Changers, and Dosimats, Metrohm Ltd., Brinkmann Instruments, Inc.
- Combination pH-reference electrode, Metrohm part no. 6.0210.100, Brinkmann Instruments, Inc.

5.12.5 Reagents

- Reverse osmosis (RO) water, ASTM Type III grade of reagent water
- Borax pH buffers, pH 4.00, pH 7.00, and pH 9.18, for electrode calibration, Beckman, Fullerton, CA.

- Calcium chloride ($CaCl_2$), 0.02 M. Dissolve 23.52 g of $CaCl_2 \cdot 2H_2O$ in RO water and dilute to 8 L.

5.12.6 Procedure

1. Use a calibrated scoop to measure ≈ 20 g of <2-mm or fine-grind, air-dry soil. If sample is moist, use calibrated scoop to achieve ≈ 20 g of air-dry soil.

2. Place the sample in a 120-mL (4-oz) paper cup.

3. Dispense 20 mL of RO water into sample and stir.

4. Place paper cup with sample in 250-mL titration beaker, allow to stand for 1 h, stirring occasionally.

5. Load beakers into sample changer.

6. Calibrate the pH meter using the pH 9.18, 7.00 and pH 4.00 buffer solutions.

7. Sample stirring, waiting interval for readings, addition of $CaCl_2$ solution, pH readings, and rinsing of electrode are controlled by computer.

8. The general sequence used by the automated system is as follows:

    - The sample is lifted so that the pH electrode is positioned above the soil sediment.
    - The sample is stirred for 30 s.
    - After 1 min, 1:1 water pH is read. Record pH to the nearest 0.01 unit.
    - The 20 mL of 0.02 M $CaCl_2$ are added to sample. The sample is stirred for 30 s.
    - After 1 min, the 1:2 $CaCl_2$ pH is read. Record pH to the nearest 0.01 unit.
    - The sample is lowered, and the electrode and stirrer are rinsed with RO water.
    - The next sample is positioned for analysis.
    - The cycle is repeated until all samples have been analyzed.

5.12.7 Calculation

No calculations are required for this procedure.

5.12.8 Reporting

Report the 1:1 water pH and the 1:2 0.01 *M* $CaCl_2$ pH to the nearest 0.1 pH unit.

5.12.9 References

Foth, H.D., and B.G. Ellis. 1988. Soil fertility. John Wiley and Sons. NY, NY. McLean, E.O. 1982. Soil pH and lime requirement. p. 199-224. In A.L. Page, R.H. Miller, and D.R. Keeney (eds.) Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Soil Survey Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys, USDA-NRCS Agric. Handb. 436. 2nd ed. U.S. Govt. Print. Office, Washington, DC.

5.13 BaCl2-Triethanolamine, pH 8.2 Extraction

5.13.1 Summary of Method

A soil sample is leached with a $BaCl_2$-TEA solution buffered at pH 8.2. Sample is allowed to stand overnight, shaken, and centrifuged. The extract is back-titrated with HCl. The difference between a blank and the extract is the extractable acidity. Extractable acidity is reported in meq 100 g^{-1} soil or (cmol (+) kg^{-1}).

5.13.2 Interferences

No significant interferences are known to exist with this method. However, for some very acid soils, the buffer capacity of the $BaCl_2$-TEA solution may be exceeded.

5.13.3 Safety

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses) when preparing reagents, especially concentrated acids and bases. Dispense concentrated acids in a fume hood. Thoroughly wash hands after handling

reagents. Use the safety showers and eyewash stations to dilute spilled acids and bases. Use sodium bicarbonate and water to neutralize and dilute spilled acids.

5.13.4 Equipment

- Electronic balance, ±1.0-mg sensitivity
- Pipettes or dispenser, adjustable volume to 40 mL
- Vortexer, mini, Analog, VRW Scientific Products
- Centrifuge tubes, 50-mL, polyethylene
- Centrifuge, Centra, GP-8, Thermo IEC, Needham Heights, MA
- Titration beakers, 250-mL, plastic, Metrohm Ltd., Brinkmann Instruments Inc.
- Automatic titrator, with control unit, sample changer, and dispenser, Metrohm Ltd., Brinkmann Instruments, Inc.
- Combination pH-reference electrode, Metrohm Ltd., Brinkmann Instruments, Inc.
- Computer, with Titrino Workcell software, Metrohom Ltd., Brinkmann Instruments, Inc., and printer

5.13.5 Reagents

- Reverse osmosis deionized (RODI) water
- Hydrochloric acid (HCl), concentrated, 12 N
- HCl, 0.13 N, standardized. Dilute 193 mL of concentrated HCl to 16-L volume with RODI water.
- Buffer solution (0.5 N BaCl2, 0.2 N Triethanolamine (TEA), pH 8.2). Dissolve 977 g of BaCl2·2H2O in 8 L of RODI water. Dissolve 477 g of TEA in 4 L of RODI water. Mix two solutions and bring to nearly 16-L volume with RODI water. Adjust to pH 8.2 with ≈ 33 mL of concentrated HCl or barium hydroxide. Bring to 16-L volume with RODI water.
- Replacement solution. Dissolve 977 g of BaCl2·2H2O in 8 L of RODI water. Add 80 mL of buffer solution and dilute to 16-L volume with RODI water.

5.13.6 Procedure

Extraction of Acidity

1. Weigh 5 g of <2-mm or fine-grind, air-dry soil to the nearest mg and place in a centrifuge tube. If sample is moist, weigh enough soil to achieve ≈ 5 g of air-dry soil. Prepare at least two reagent blanks (no sample in tube) and one quality control check sample per 21 samples.

2. Add 40.00 mL of $BaCl_2$-TEA solution to sample. Cap the tube and shake to ensure all soil is wetted. Place tube in a rack.

3. Place tube rack on its side and gently shake to stratify the mixture lengthwise along the tube. Allow to stand overnight on its side.

4. Centrifuge sample at 2000 rpm for 5 min.

5. Decant extract into numbered titration beakers.

6. Add 40 mL of replacement solution to sample.

7. Cap tube and use a vortexer to loosen soil. Manually shake.

8. Repeat Sections 7.4 – 7.7.

9. Repeat Sections 7.4 – 7.5. Total volume in titration beaker should be ≈ 120 mL.

Titration of BaCl2-TEA Extract

10. Place titration beakers on automatic sample changer.

11. Refer to the manufacturer's manual for operation of the automatic titrator.

12. Calibrate the titrator meter with 9.18, 7.00 and 4.00 pH buffers. Set-up the automatic titrator to sent end point mode. The "Set" pH parameters are listed as follows:

Parameter	Value
Ep1	pH 4.60
Dyn change pH	1.5 units
Drift	0.4 mV s-1
Time delay	10 s
Drift	0.4 mV s-1
Temp	25°C
Stop Volume	75 mL

13. If pre-titration pH is 0.3 units lower than the average pH of the blanks, re-run using a 0.5-g sample.

14. Record the titer to the nearest 0.01 mL. Record the normality of the HCl solution. Average the titer of the reagent blanks and record.

5.13.7 Calculations

$$\text{Extractable acidity (meq 100 g}^{-1}) = \{[(B - T) \times N \times R]/C\} \times 100$$

where:

- B = Average reagent blank titer (mL)
- T = Sample titer (mL)
- *N* = Normality of HCl
- C = Sample Weight (g)
- 100 = Conversion factor (100-g basis)
- R = Air-dry/oven-dry ratio (procedure 3D1) or field-moist/oven-dry ratio (procedure 3D2)

5.13.8 Report

Report extractable acidity to the nearest 0.1 meq 100 g^{-1} (cmol (+) kg^{-1}).

5.13.9 Precision and Accuracy

Precision and accuracy data are available from the SSL upon request.

5.13.10 References

Holmgren, G.G.S., R.L. Juve, and R.C. Geschwender. 1977. A mechanically controlled variable rate leaching device. Soil Sci. Am. J. 41:1207-1208.

5.14 Cation Exchange Capacity (Total)

5.14.1 Summary of Method

Displacement after washing is the basis for this procedure. The CEC is determined by saturating the exchange sites with an index cation (NH_4^+); washing the soil free of excess saturated salt; displacing the index cation (NH_4^+) adsorbed by the soil; and measuring the amount of the index cation (NH_4^+). A sample is leached using 1 *N* NH_4OAc and a mechanical vacuum extractor (Holmgren et al., 1977). The extract is weighed and saved for analyses of the cations. The NH_4^+ saturated soil is rinsed with ethanol to remove the NH_4^+ that was not adsorbed. The soil is then rinsed with 2 *M* KCl. This leachate is then analyzed by steam distillation and titration to determine the NH_4^+ adsorbed on the soil exchange complex. The CEC by NH_4OAc, pH 7 is reported in meq 100 g^{-1} or (cmol (+) kg^{-1}) soil in procedure 4B1a1a1a1.

5.14.2 Interferences

Incomplete saturation of the soil with NH_4^+ and insufficient removal of NH_4^+ are the greatest interferences to this method. Ethanol removes some adsorbed NH_4^+ from the exchange sites of some soils. Isopropanol rinses have been used for some soils in which ethanol removes adsorbed NH_4^+. Soils that contain large amounts of vermiculite can irreversibly "fix" NH_4^+. Soils that contain large amounts of soluble carbonates can change the extractant pH and/or can contribute to erroneously high cation levels in the extract. This method overestimates the “field” CEC of soils with pH <7 (Summer and Miller, 1996).

5.14.3 Safety

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses) when preparing reagents, especially concentrated acids and bases. Dispense concentrated acids and bases in a fume hood. Thoroughly wash hands after handling reagents. Use the safety showers and eyewash stations to dilute spilled acids and

bases. Use sodium bicarbonate and water to neutralize and dilute spilled acids. Nessler's reagent contains mercury which is toxic. Proper disposal of the Nessler's reagent and clean-up of equipment in contact with the reagent is necessary.

Ethanol is flammable. Avoid open flames and sparks. Standard laboratory equipment includes fire blankets and extinguishers for use when necessary. Follow the manufacturer's safety precautions when using the vacuum extractor and the Kjeltec Auto Analyzers.

5.14.4 Equipment

- Electronic balance, ±1.0-mg sensitivity
- Mechanical vacuum extractor, 24-place, SAMPLETEX, MAVCO Industries, Lincoln, NE (Fig. 1 and 2)
- Tubes, 60-mL, polypropylene, for extraction (0.45-μm filter), reservoir, and tared extraction tubes
- Rubber tubing, 3.2 ID x 1.6 OD x 6.4 mm (1/8 ID x 1/16 OD x 1 in) for connecting syringe barrels.
- Kjeltec Auto 2300 Sampler System, Tecator, Perstorp Analytical
- Digestion tubes, straight neck, 250 mL
- Syringe filters, 0.45 μm, Whatman
- Wash bottles
- Vials, plastic
- Centrifuge, Centra, GP-8, Thermo IEC, Needham Heights, MA

5.14.5 Reagents

- Reverse osmosis deionized (RODI) water, ASTM Type I grade of reagent water
- Ammonium acetate solution (NH_4OAc), 1 N, pH 7.0. Add 1026 mL of glacial acetic acid (CH_3COOH) to 15 L RODI water. Add 1224 mL of concentrated ammonium hydroxide (NH_4OH). Cool. Allow to stand one day to equilibrate to room temperature. Mix and adjust to pH 7.0 with CH_3COOH (typically, ≈ 40 mL) or NH_4OH and dilute with RODI water to 18 L.
- Ethanol (CH_3CH_2OH), 95%, U.S.P.
- Nessler's reagent. Add 4.56 g of potassium iodide (KI) to 30 mL RODI water. Add 5.68 g of mercuric iodide (HgI_2). Stir until dissolved. Dissolve 10 g of sodium hydroxide (NaOH) in 200 mL of RODI water. Transfer NaOH solution to a 250-mL volumetric flask and slowly add K-Hg-I solution. Dilute to volume with RODI water and thoroughly mix. Solution

should not contain a precipitate. Solution can be used immediately. Store in brown bottle to protect from light.

- Potassium chloride solution, 2 M. Add 1341.9 g of KCl reagent in 8 L RODI water. Allow solution to equilibrate to room temperature. Dilute to 9 L with RODI water.
- Boric acid, 4% (w:v), with bromcresol green-methyl red indicator (0.075 % bromcresol green and 0.05% methyl red), Chempure.
- Hydrochloric acid (HCl), 0.1 N, standardized. Dilute 167 mL of concentrated HCl in 20 L of RODI water. Refer to procedure for Standardization of Acids.
- NaOH, 1 M. Add 500 mL of 50% NaOH solution to 8 L of RODI water. Dilute to 9 L with RODI water.

5.14.6 Procedure

Extraction of Bases

1. Weigh 2.5 g of <2-mm, air-dry soil to the nearest mg and place in a labeled extraction tube (ET). If sample is fine-grind, weigh 1 g to the nearest mg. If sample is moist, weigh enough soil to achieve ≈ 2.5 or 1g, respectively, of air-dry soil. Prepare one quality control check sample per 24 samples.

2. Place labeled ET on extractor and connect to corresponding tared extraction tube ($TETNH_4OAc$) with rubber tubing.

3. Use wash bottle to rinse inside of ET with NH_4OAc. All soil should be wetted and be sure there are no air bubbles. Shaking, swirling, or stirring may be required to wet organic samples. Fill ET to the 20-mL mark with NH_4OAc solution (≈ 10 mL).

4. Secure reservoir tube (RT) to top of ET tube and let stand for 30 min. Extract at 30-min rate the NH_4OAc solution until 2 mL of this solution remains above soil level. Turn off extractor. Do not let soil dry.

5. Add 40 mL of NH_4OAc solution to the RT. Set extractor for an overnight (12h) extraction. Extractor will turn off automatically.

6. Next day, remove RT from top of extractor and place in a clean container. Carefully remove $TETNH_4OAc$. Leave the rubber tubing on the ET. Weigh each $TETNH_4OAc$ containing the NH_4OAc extract to the nearest mg.

7. Mix the extract in each TETNH_4OAc by manually shaking. Fill a labeled plastic vial with extract solution and cap. Discard the excess properly. The solution in the vial is reserved for analyses of extracted cations (procedure 4B1a1b1-4) on the atomic absorption spectrophotometer (AAS). Some samples may be cloudy and need to be filtered prior to analysis on the AA. If extracts are not to be determined immediately after collection, then store samples at 4°C in plastic tubes.

Removal of Excess Ammonium Acetate

8. Re-connect the TETNH_4OAc with paired ET. Use a wash bottle to rinse the sides of the ET with ethanol to remove any remaining NH_4OAc or soil particles adhering to the ET. All soil should be wetted and no air bubbles. Fill ET to the 20-mL mark with ethanol. Secure RT to top of ET tube and let stand for 30 min.

9. Extract at the 30-min extraction rate the ethanol solution until 2 mL of this solution remains above the soil level. Turn off the extractor. Do not let soil dry.

10. Add 45 mL of ethanol to the RT. Extract (≈ 45 min) the ethanol until 2 mL of this solution remains above the soil level. Turn off the extractor. Do not let soil dry. Disconnect the TETNH_4OAc from the ET and discard the ethanol properly.

11. Re-connect the TETNH_4OAc to the ET and add 55 mL of ethanol to the RT. Set the extractor for 45 min. Turn off the extractor. Remove the TETNH_4OAc, leaving the tubing connected to the ET. Discard the ethanol properly.

12. After the final ethanol wash, collect a few drops of ethanol extract from the ET on a spot plate. Test for NH_4+ by using Nessler's reagent. A yellow, red to reddish brown precipitate is a positive test. If the test is positive, repeat the ethanol wash and retest with Nessler's reagent. Repeat until a negative test is obtained.

2 M KCl Rinse

13. Connect a new labeled extraction tube (ETKCl) with rubber tubing to ET on extractor.

14. Use a wash bottle to rinse inside of ET with 2 M KCl to remove any remaining ethanol or soil particles adhering to the ET. All soil should be wetted and no air bubbles. Fill ET to the 20-mL mark with KCl solution and let stand for 30.

15. Extract at the 30-min rate the KCl solution until 2 mL of this solution remains above soil level. Turn off extractor. Do not let soil dry.

16. Secure RT to top of ET tube. Add 40 mL KCl solution to RT and set the extract for 45 min. Remove the ET and ETKCl from the extractor.

Steam Distillation: Setup, Operation, and Analysis

17. Transfer the contents of the ETKCl to a 250-mL digestion tube. If extracts are not to be determined immediately after collection, then store samples at 4°C.

18. Refer to the manufacturer's manual for operation of the distillation unit. The following are only very general guidelines for instrument conditions.

Program: Kjeldahl 1
Receiving solution (boric acid): 30 mL
Water: 0 mL
Alkali (NaOH): 20 mL
Mode: Delay
Time: 1 s
Distillation: Volume
Tube Drain: Yes

19. When using new reagents, e.g., boric acid, reagent blanks are distilled in 2 sets of 6, one set per Kjeltec machine. Each set of 6 is averaged and recorded on bench worksheet and manually set on each machine. During the steam distillation, the mean reagent blank titer is automatically subtracted from the sample titer.

20. Record the normality of standardized acid.

21. Connect the tube to the distillation unit. Close the safety door. Distillation and titration are performed automatically. Record the titer in mL of titrant.

5.14.7 Calculations

$$CEC = [Titer \times N \times 100 \times R]/[Sample\ Weight\ (g)]$$

where:

CEC = Cation Exchange Capacity (meq 100 g^{-1})
Titer = Titer of sample (mL)
N = Normality of HCl titrant

100 = Conversion factor to 100-g basis
R = Air-dry/oven-dry ratio (procedure 3D1) or field-moist/oven-dry ratio (procedure 3D2)

5.14.8 Report

Report CEC-7 to the nearest 0.1 meq 100 g^{-1} (cmol (+) kg^{-1}).

5.14.9 References

Holmgren, G.G.S., R.L. Juve, and R.C. Geschwender. 1977. A mechanically controlled variable rate leaching device. Soil Sci. Am. J. 41:1207-1208.

Peech, M., L.T. Alexander, L.A. Dean, and J.F. Reed. 1947. Methods of soil analysis for soil fertility investigations. USDA Circ. 757, 25 pp.

Sumner, M.E., and W.P. Miller. 1996. Cation exchange capacity and exchange coefficients. p. 1201-1229. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. No. 5. ASA and SSSA, Madison, WI.

5.15 Cation Exchange Capacity (Ca, Mg, K, Na)

5.15.1 Summary of Method

An NH_4OAc extract (from section 5.13) is diluted with an ionization suppressant (La_2O_3). The analytes are measured by an atomic absorption spectrophotometer (AAS). The analyte is measured by absorption of the light from a hollow cathode lamp. An automatic sample changer is used to aspirate a series of samples. The AAS converts absorption to analyte concentration. Data are automatically recorded by a microcomputer and printer. The NH_4OAc extracted cations, Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, are reported in meq 100 g^{-1} soil or (cmol (+) kg^{-1}).

5.15.2 Interferences

There are four types of interferences (matrix, spectral, chemical, and ionization) in the analyses of these cations. These interferences vary in importance, depending upon the particular analyte selected. Do not use borosilicate tubes because of potential leaching of analytes.

5.15.3 Precautions

Wear protective clothing and safety glasses. When preparing reagents, exercise special care. Restrict the use of concentrated HCl to a fume hood. Many metal salts are extremely toxic and may be fatal if ingested. Thoroughly wash hands after handling these metal salts.
Follow standard laboratory procedures when handling compressed gases. Gas cylinders should be chained or bolted in an upright position. Acetylene gas is highly flammable. Avoid open flames and sparks. Standard laboratory equipment includes fire blankets and extinguishers for use when necessary. Follow the manufacturer's safety precautions when using the AAS.

5.15.4 Equipment/Materials

- Electronic balance, ±1.0-mg sensitivity
- Atomic absorption spectrophotometer (AAS), double-beam optical system, AAnalyst, 300, Perkin-Elmer Corp., Norwalk, CT
- Autosampler, AS-90, Perkin-Elmer Corp., Norwalk, CT
- Computer, with AA WinLab software, Perkin-Elmer Corp., Norwalk, CT, and printer
- Single-stage regulator, acetylene
- Digital diluter/dispenser, with syringes 10000 and 1000 µL, gas tight, MicroLab 500, Hamilton Co., Reno, NV
- Plastic test tubes, 15-mL, 16 mm x 100, for sample dilution and sample changer
- Containers, polyethylene
- Peristaltic pump

5.15.5 Reagents

- Reverse osmosis deionized (RODI) water, ASTM Type I grade of reagent water
- Hydrochloric acid (HCl), concentrated 12 N
- HCl, 1:1 HCl:RODI, 6 N. Carefully mix 1 part of concentrated HCl to 1 part RODI water.
- NH_4OH, reagent-grade, specific gravity 0.90

- Glacial acetic acid, 99.5%
- Ammonium acetate solution (NH_4OAc), 1 N, pH 7.0. Add 1026 mL of glacial acetic acid (CH_3COOH) to 15 L RODI water. Add 1224 mL of concentrated ammonium hydroxide (NH_4OH). Cool. Allow to stand one day to equilibrate to room temperature. Mix and adjust to pH 7.0 with CH_3COOH (typically, ≈ 40 mL) or NH_4OH and dilute with RODI water to 18 L. The NH_4OAc solution is used for extraction of cations (procedure 4B1a1).
- NH_4OAc solution, 2.0 N, pH 7.0. Mix 228 mL of glacial acetic acid in 1200 mL of RODI water. While stirring, carefully add 272 mL of concentrated NH_4OH. Cool. Allow to stand one day to equilibrate to room temperature. Mix and adjust pH 7.0 using CH_3COOH or NH_4OH. Dilute to 2 L with RODI water.
- Stock lanthanum ionization suppressant solution (SLISS), 65,000 mg L-1. Wet 152.4 g lanthanum oxide (La_2O_3) with 100 mL RODI water. Slowly and cautiously add 500 mL of 6 N HCl to dissolve the La_2O_3. Cooling the solution is necessary. Dilute to 2 L with RODI water. Filter solution. Store in polyethylene container.
- Working lanthanum ionization suppressant solution (WLISS), 2000 mg L-1. Dilute 61.5 mL of SLISS with 1800 mL of RODI water (1:10). Dilute to final volume of 2-L with RODI water. Store in polyethylene container.
- Primary stock standards solutions (PSSS), high purity, 1000 mg L-1: Ca, Mg, K, and Na.
- Working stock mixed standards solution (WSMSS), High, Medium, Low, Very Low, and Blank. In five 500-mL volumetric flasks, add 250 mL of 2 N NH_4 OAc and the following designated amounts of Ca PSSS, Mg PSSS, K PSSS, and Na PSSS. Dilute to volume with RODI. Invert to thoroughly mix. Store in polyethylene containers. Prepare fresh weekly. Store in the refrigerator. Allow to equilibrate to room temperature before use. Prepare WSMSS as follows:

    - High Standard WSMSS: 90 mL Ca PSSS, 7.5 mL Mg PSSS, 20.0 mL K PSSS, and 100.0 mL Na PSSS = 180 mg L^{-1} Ca, 15 mg L^{-1} Mg, 40 mg L^{-1} K, and 200 mg L^{-1} Na.
    - Medium Standard WSMSS: 60 mL Ca PSSS, 5.0 mL Mg PSSS, 10.0 mL K PSSS, and 50.0 mL Na PSSS = 120 mg L^{-1} Ca, 10 mg L^{-1} Mg, 20 mg L^{-1} K, and 100 mg L^{-1} Na.
    - Low Standard WSMSS: 30 mL Ca PSSS, 2.5 mL Mg PSSS, 5.0 mL K PSSS, and 10.0 mL Na PSSS = 60 mg L^{-1} Ca, 5 mg L^{-1} Mg, 10 mg L^{-1} K, and 20 mg L^{-1} Na.
    - Low/Low Standard WSMSS: 12.5 mL Ca PSSS, 0.25 mL Mg PSSS, 0.125 mL K PSSS, and 5.0 mL Na PSSS = 25 mg L^{-1} Ca, 0.5 mg L^{-1} Mg, 0.25 mg L^{-1} K, and 10 mg L^{-1} Na.
    - Blank WSMSS = 0 mL of Ca, Mg, K, and Na PSSS.

- Mixed calibration standard solutions (MCSS), High, Medium, Low, Very Low, and Blank. Dilute 1 part WSMSS with 19 parts of WLISS (1:20) dilution with resulting concentrations for MCSS as follows:

- MCSS High Standard: 9.0 mg L^{-1} Ca, 0.75 mg L^{-1} Mg, 2.0 mg L^{-1} K, and 10.0 mg L^{-1} Na.
- MCSS Medium Standard: 6.0 mg L^{-1} Ca, 0.5 mg L^{-1} Mg, 1.0 mg L^{-1} K, and 5.0 mg L^{-1} Na.
- MCSS Low Standard: 3.0 mg L^{-1} Ca, 0.25 mg L^{-1} Mg, 0.5 mg L^{-1} K, and 1.0 mg L^{-1} Na.
- MCSS Very Low Standard: 1.25 mg L^{-1} Ca, 0.025 mg L^{-1} Mg, 0.125 K, and 0.5 mg L^{-1} Na.
- Blank = 0 mg L^{-1} Ca, Mg, K, and Na.

- Compressed air with water and oil traps.
- Acetylene gas, purity 99.6%.

5.15.6 Procedure

Dilution of Calibration Standards and Sample Extracts

1. The 10-mL syringe is for dilutent (WLISS). The 1-mL syringe is for the MCSS and NH_4OAc extracts (procedure 5.13). Set the digital diluter at a 1:20 dilution. See reagents for preparation of the MCSS (High, Medium, Low, Very Low, Blank). Dilute 1 part NH_4OAc sample extract with 19 parts of WLISS (1:20 dilution).

2. Dispense the diluted sample solutions into test tubes which have been placed in the sample holders of the sample changer.

AAS Set-up and Operation

Refer to the manufacturer's manual for operation of the AAS. The following are only very general guidelines for instrument conditions for the various analytes.

Analyte	Conc. (mg L^{-1})	Burner & angle	Wavelength (nm)	Slit (mm)	Fuel/Oxidant (C_2H_2/Air)
Ca	9.0	10 cm @ 0°	422.7	0.7	1.5/10.0
Mg	0.75	10 cm @ 0°	285.2	0.7	1.5/10.0
K	2.0	10 cm @ 0°	766.5	0.7	1.5/10.0
Na	10.0	10 cm @ 30°	589.0	0.2	1.5/10.0

3. Use the computer and printer to set instrument parameters and to collect and record instrument readings.

AAS Calibration and Analysis

4. Calibrate the instrument by using the MCSS (High, Medium, Low, Very Low, Blank). The data system will then associate the concentrations with the instrument responses for each MCSS. Rejection criteria for MCSS, if R2 <0.99.

5. If sample exceeds calibration standard, the sample is diluted 1:5, 1:20, 1:100, etc., with 1 N NH_4OAc followed by 1:20 dilution with WLISS.

6. Perform one quality control (QC) (Low Standard MCSS) every 12 samples. If reading is not within 10%, the instrument is re-calibrated and QC re-analyzed.

7. Record analyte readings to 0.01 unit.

5.15.7 Calculation

The instrument readings for analyte concentration are in mg L^{-1}. These analyte concentrations are converted to meq 100 g^{-1} as follows:

Soil Analyte Concentration (meq 100 g^{-1}) = $[A \times [(B_1 - B_2)/B_3] \times C \times R \times 100]/[1000 \times E \times F]$

where:

A = Analyte (Ca, Mg, K, Na) concentration in extract (mg L^{-1})
B_1 = Weight of extraction syringe and extract (g)
B_2 = Weight of tared extraction syringe (g)
B_3 = Density of 1 *N* NH_4OAc at 20°C (1.0124 g cm-3)
C = Dilution, if performed
100 = Conversion factor (100-g basis)
R = Air-dry/oven-dry ratio (procedure 3D1) or field-moist/oven-dry ratio (procedure 3D2)
1000 = mL L^{-1}
E = Soil sample weight (g)
F = Equivalent weight (mg meq^{-1})

where:

Ca^{+2} = 20.04 mg meq^{-1}
Mg^{+2} = 12.15 mg meq^{-1}
Na^{+1} = 22.99 mg meq^{-1}
K^{+1} = 39.10 mg meq^{-1}

5.15.8 Reporting

Report the extractable Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} to the nearest 0.1 meq 100 g^{-1} (cmol (+) kg^{-1}).

5.15.9 References

Thomas, G.W. 1982. Exchangeable cations. p. 159-165. In A.L. Page, R.H. Miller, and D.R. Keeney (eds.) Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

5.16 Acid Oxalate Extraction (Al, Fe, Mn, P, Si)

5.16.1 Summary of Method

A soil sample is extracted with a mechanical vacuum extractor (Holmgren et al., 1977) in a 0.2 *M* ammonium oxalate solution buffered at pH 3.0 under darkness. The ammonium oxalate extract is weighed. The ammonium oxalate extract is diluted with reverse osmosis deionized water. The analytes are measured by a inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Data are automatically recorded by a computer and printer. All these data are reported in percent except Mn and P, which are reported in mg kg^{-1}.

5.16.2 Interferences

There are four types of interferences (matrix, spectral, chemical, and ionization) in the ICP analyses of these elements. These interferences vary in importance, depending upon the particular analyte chosen.

The ammonium oxalate buffer extraction is sensitive to light, especially UV light. The exclusion of light reduces the dissolution effect of crystalline oxides and clay minerals. If the sample contains large amounts of amorphous material (>2% Al), an alternate method should be used, i.e., shaking with 0.275 *M* ammonium oxalate, pH 3.25, 1:100 soil:extractant.

5.16.3 Precautions

Wear protective clothing and eye protection. When preparing reagents, exercise special care. Follow standard laboratory practices when handling compressed gases. Gas cylinders should be chained or bolted in an upright position. Follow the manufacturer's safety precautions when using the UV spectrophotometer and ICP.

5.16.4 Equipment/Materials

- Electronic balance, ±1.0-mg sensitivity
- Mechanical vacuum extractor, 24 place, SAMPLETEX, MAVCO Industries, Lincoln, NE
- Tubes, 60-mL, polypropylene, for extraction (0.45-µm filter), reservoir, and tared extraction tubes
- Rubber tubing, 3.2 ID x 1.6 OD x 6.4 mm, (1/8 ID x 1/16 OD x 1 in) for connecting syringe barrels
- Dispenser, 30-mL
- Pipettes, electronic digital, 10000 and 1000 µL, with tips 10000 and 1000 µL
- Containers, polyethylene
- Inductively coupled plasma atomic emission spectrophotometer (ICP-AES), dual-view, with high-solids nebulizer, alumina or quartz injector, Optima 4300 DV, Perkin-Elmer Corp., Norwalk, CT.
- Automsampler, AS-93, Perkin-Elmer Corp., Norwalk, CT
- Computer, with WinLab32TM software, and printer
- Single-stage regulator, high-purity, high-flow, argon
- Test tubes, 15-mL, 16 mm x 100, for sample dilution and sample changer, Fisher Scientific
- Vortexer, mini, MV1, VWR Scientific Products
- Spectrophotometer, UV-Visible, Varian, Cary 50 Conc, Varian Australia Pty Ltd.
- Computer with Cary WinUV software, Varian Australia Pty Ltd., and printer
- Cuvettes, plastic, 4.5-mL, 1-cm light path, Daigger Scientific

5.16.5 Reagents

- Reverse osmosis deionized (RODI) water, ASTM Type I grade of reagent water
- Ammonium oxalate buffer solution, 0.2 M, pH 3.0. Solution A (base): Dissolve 284 g of $(NH_4)2C_2O_4 \bullet 2H_2O$ in 10 L of DI water. Solution B (acid): Dissolve 252 g of $H_2C_2O_4.H_2O$ in 10 L of DI water. Mix 4 parts solution A with 3 parts solution B. Adjust ammonium oxalate solution pH by adding either acid or base solution. Store in a polypropylene bottle.

- Borax pH buffers, pH 4.00, 7.00, and 9.18 for electrode calibration, Beckman, Fullerton, CA.
- Primary Fe standard, 1000 mg L-1. Certified Reference Solution, Fisher Chemical Scientific Co., Fairlawn, N.J.
- Primary Al standard, 1000 mg L-1. Certified Reference Solution, Fisher Chemical Scientific Co., Fairlawn, N.J.
- Primary Si standard, 1000 mg L-1. Certified Reference Solution, Fisher Chemical Scientific Co., Fairlawn, N.J.
- Primary Mn standard, 1000 mg L-1. Certified Reference Solution, Fisher Chemical Scientific Co., Fairlawn, N.J.
- Primary P standard, 1000 mg L-1. Certified Reference Solution, Fisher Chemical Scientific Co., Fairlawn, N.J.
- High mixed calibration standard. Mix 60 mL of each primary standard (Si, Fe, and Al) with 10 mL of primary Mn standard and 20 mL of primary P standard in 1 L volumetric flask. Add 100 mL of 0.2 M ammonium oxalate solution and make to 1-L volume with RODI water. The elements are added in the order (Si, Fe, Al, Mn, and P) to avoid element precipitation. Resulting solution contains 60 mg L-1 each of Si, Fe, and Al, 10 mg L-1 Mn, and 20 mg L-1 P. Invert to mix thoroughly. Store in a polyethylene bottle. Make fresh weekly. Store in a refrigerator.
- Medium mixed calibration standard. Mix 30 mL of each primary standard (Si, Fe, and Al) with 5 mL of primary Mn standard and 10 mL of primary P standard in 1 L volumetric flask. Add 100 mL of 0.2 M ammonium oxalate solution and make to 1-L volume with RODI water. The elements are added in the order (Si, Fe, Al, Mn, and P) to avoid element precipitation. Resulting solution contains 30 mg L-1 each of Si, Fe, and Al, 5 mg L-1 Mn, and 10 mg L-1 P. Invert to mix thoroughly. Store in a polyethylene bottle. Make fresh weekly. Store in a refrigerator.
- Low mixed calibration standard. Mix 10 mL of each primary standard (Si, Fe, and Al) with 2 mL of primary Mn standard, and 3 mL primary P standard in 1 L volumetric flask. Add 100 mL of 0.2 M ammonium oxalate solution and make to 1-L volume with RODI water. The elements are added in the order (Si, Fe, Al, Mn, and P) to avoid element precipitation. Resulting solution contains 10 mg L-1 each of Si, Fe, and Al, 2 mg L-1 Mn, and 3 mg L-1 P. Invert to mix thoroughly. Store in a polyethylene bottle. Make fresh weekly. Store in a refrigerator.
- Low Si calibration standard. Mix 5 mL of Si primary standard in 1-L volumetric flask. Add 100 mL of 0.2 M ammonium oxalate solution and make to 1-L volume with RODI water. Resulting solution contains 5 mg L-1 Si. Invert to mix thoroughly. Store in polyethylene bottle. Make fresh weekly. Store in a refrigerator.
- Very Low Si calibration standard. Mix 2 mL of Si primary standard in 1-L volumetric flask. Add 100 mL of 0.2 M ammonium oxalate solution and make to 1-L volume with RODI

water. Resulting solution contains 2 mg L-1 Si. Invert to mix thoroughly. Store in polyethylene bottle. Make fresh weekly. Store in a refrigerator.

- Calibration reagent blank solution. Add 100 mL of 0.2 M ammonium oxalate solution and make to 1-L volume with RODI water. Store in polyethylene bottle. Make fresh weekly. Store in a refrigerator.
- Argon gas, purity 99.9%
- Nitrogen, purity 99.9%

5.16.6 Procedure

Extraction of Fe, Mn, Al, Si, and P

Weigh 0.5 g of <2-mm, air-dry or fine-grind soil the nearest mg and place in sample tube. If sample is moist, weigh enough soil to achieve ≈ 0.5 g. Prepare two reagent blanks (no sample in tube) per set of 48 samples.

1. Place labeled ET on extractor and connect to corresponding tared extraction tube (TETOxalate) with rubber tubing.

2. Use a dispenser to add 15.00 mL of ammonium oxalate buffer to the ET. Make sure that the sample is thoroughly wetted. During the addition, wash sides of the tube and wet the sample. Shaking, swirling, or stirring may be required to wet organic samples. Allow sample to stand for at least 30 min. Cover samples with black plastic bag to exclude light.

3. Secure reservoir tube (RT) to top of ET tube. Set extractor for 30-min extraction rate and extract until the ammonium oxalate buffer solution is at a 0.5 to 1.0-cm height above sample. Turn off extractor.

4. Add 35 mL of ammonium oxalate buffer to the RT.

5. Cover the extractor with a black plastic bag to exclude light. Adjust the extraction rate for a 12-h extraction.

6. After the extraction, shut off the extractor. Carefully remove TETOxalate. Leave the rubber tubing on the ET.

7. Weigh each syringe containing ammonium oxalate extract to the nearest mg.

8. Mix extract in each TETOxalate by manually shaking. Fill a disposable tube with extract solution. This solution is reserved for determinations of Fe, Mn, Al, Si, and P. If optical density is to be measured, fill a disposable cuvette with extract solution. Discard excess solution properly. If extracts are not to be determined immediately after collection, then store samples at 4°C.

Determination of Optical Density of Extract

9. Place 4 mL of ammonium oxalate extract in disposable cuvette.

10. Place 4 mL of ammonium oxalate reagent blank in disposable cuvette.

11. On the spectrophotometer, select a 430-nm wavelength. Select normal slit width and height. Refer to manufacturer's manual for operation of the spectrophotometer.

12. Use the ammonium oxalate extract reagent blank to zero spectrophotometer.

13. Record optical density of ammonium oxalate extract to nearest 0.000 unit.

Dilution of Sample Extracts and Standards

14. Dilute ammonium oxalate extracts (1:10) with RODI water. Add 1 part ammonium oxalate sample extract with 9 parts dilution solution. Pipet 0.7 mL of extract and 6.3 mL RODI water. Vortex. Calibration reagent blanks and calibration standards are not diluted.

15. Dispense the diluted solutions into test tubes that have been placed in the sample holder of the sample changer.

ICP-AES Set-up and Operation

16. Refer to the manufacturer's manual for operation of the ICP-AES. The following parameters are only very general guidelines for instrument conditions for the various analytes.

Parameter	Value
Plasma	
Source Equilibration Delay	20 sec
Plasma Aerosol Type	Wet
Nebulizer Start-up Conditions	Gradual
Plasma	15 L min^{-1}

Auxiliary	0.5 L min^{-1}
Nebulizer	0.85 L min^{-1}
Power	1450 Watts
View Dist	15.0
Plasma View	Radial
Peristaltic Pump	
Sample Flow Rate	2.00 L min^{-1}
Sample Flush Time	35 sec
Wash Parameters	Between samples
Wash Rate	2.00 mL min^{-1}
Wash Time	30 sec
Background Correction	2 point (all elements)
Read Delay	2 sec
Replicates	2

Nebulizer pressure depends on the type of nebulizer that is being used, i.e., low flow nebulizer requires a higher nebulizer pressure whereas a higher flow nebulizer requires a lower nebulizer pressure. To check for correct nebulizer pressure, aspirate with 1000.0 mg L^{-1} yttrium. Adjust pressure to correct yttrium bullet.

17. Analyte data are reported at the following wavelengths:

Element	Wavelength (nm)
Fe	259.94
Al	308.22
Si	251.61
Mn	257.61
P	213.61

18. Use the computer and printer to set instrument parameters and to collect and record instrument readings. The instrument readings are programmed in mg L^{-1}.

ICP-AES Calibration and Analysis

19. Use a multipoint calibration for ICP-AES analysis of ammonium oxalate extracts. The ICP calibrates the blank first, low standard, medium standard, followed by the high standard. Prepare a quality control (QC) standard with analyte concentration between the high and low calibration standards. The ICP reads the QC after the high standard. If the QC falls within the range set by operator (±10%), the instrument proceeds to analyze the unknowns.

If the QC is outside the range, the instrument restandardizes. The QC is analyzed approximately every 12 samples.

20. If sample exceeds calibration standard, dilute 1:5 (1 mL of sample extract with 4 mL 0.02 M ammonium oxalate extracting solution), followed by a 1:10 (1 mL of 1:5 solution with 9 mL RODI water). This makes for a 1:50 dilution.

21. Record analyte readings to the nearest 0.01 unit.

5.16.7 Calculation

The instrument readings are the analyte concentration (mg L^{-1} Fe, Mn, Al, Si, and P). Use these values to calculate the analyte concentration in percent in the soil for Fe, Al, and Si and mg kg^{-1} for Mn and P as follows:

$$\text{Soil Fe, Al, Si (\%)} = [A \times [(B1 - B2)/B3] \times C1 \times C2 \times R \times 100]/(E \times 1000 \times 1000)$$

where:

A = Sample extract reading (mg L^{-1})
B_1 = Weight of syringe + extract (g)
B_2 = Tare weight of syringe (g)
B_3 = Density of 0.2 *M* ammonium oxalate solution at 20° C (1.007 g mL^{-1})
C_1 = Dilution, required
C_2 = Dilution, if performed
R = Air-dry/oven-dry ratio or field-moist/oven-dry ratio
E = Sample weight (g)
100 = Conversion factor to 100-g basis
1000 = Factor in denominator (mL L^{-1})
1000 = Factor in denominator (mg g^{-1})

$$\text{Soil Mn, P (mg kg}^{-1}) = [\, A \times [(B_1 - B_2)/B_3] \times C_1 \times C_2 \times R \times 1000]/(E \times 1000)$$

where:

A = Sample extract reading (mg L^{-1})
B_1 = Weight of syringe + extract (g)
B_2 = Tare weight of syringe (g)
B_3 = Density of 0.2 *M* ammonium oxalate solution at 20° C (1.007 g mL^{-1})
C_1 = Dilution, required
C_2 = Dilution, if performed

R = Air-dry/oven-dry ratio or field-moist/oven-dry ratio
1000 = Conversion factor in numerator to kg-basis
1000 = Factor in denominator (mL L^{-1})
E = Sample weight (g)

5.16.8 Reporting

Report the percent ammonium oxalate extractable Al, Fe, and Si to the nearest 0.01%. Report the concentration of ammonium oxalate extractable Mn and P to the nearest mg kg^{-1} soil. Report the optical density of the ammonium oxalate extract to the nearest 0.01 unit.

5.16.9 References

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Holmgren, G.G.S., R.L. Juve, and R.C. Geschwender. 1977. A mechanically controlled variable rate leaching device. Soil Sci. Am. J. 41:1207-1208.

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Mehra, O.P., and M.L. Jackson. 1960. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. Clays Clay Miner. 7:317-327.

Soil Survey Staff. 1999. Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys. 2nd ed. USDA-NRCS. Govt. Print. Office, Washington DC.

Wada, K. 1989. Allophane and imogolite. p. 1051-1087. In J.B. Dixon and S.B. Weed (eds.). Minerals in soil environment. 2nd ed. Soil Sci. Soc. Am. Book Series No. 1. ASA and SSSA, Madison.

5.17 Electrical Conductivity (Saline Prediction)

5.17.1 Summary of Method

A soil sample is mixed with water and allowed to stand overnight. The electrical conductivity (EC) of the mixture is measured using an electronic bridge. The EC by this method (4F1a1a1) is used to indicate the presence of soluble salts (U.S. Salinity Laboratory Staff, 1954).

5.17.2 Interferences

Reverse osmosis deionized water is used to zero and flush the conductivity cell. The extract temperature is assumed to be 25°C. If the temperature deviates significantly, a correction may be required.

Provide airtight storage of KCl solution and samples to prevent soil release of alkali-earth cations. Exposure to air can cause gains and losses of water and dissolved gases significantly affecting EC readings.

5.17.3 Precautions

No significant hazards are associated with this procedure. Follow standard laboratory safety practices.

5.17.4 Equipment/Materials

- Electronic balance, ±1.0-mg sensitivity
- Conductivity bridge and conductivity cell, with automatic temperature adjustment, 25 ± 0.1°C, Markson Model 1056, Amber Science, Eugene, OR
- Plastic cups, 30 mL (1 fl. oz.), with lids, Sweetheart Cup Co. Inc., Owings Mills, MD
- Dispenser, Repipet or equivalent, 0 to 10 mL

5.17.5 Reagents

- Reverse osmosis (RO) water, ASTM Type III grade of reagent water

- Potassium chloride (KCl), 0.010 N. Dry KCl overnight in oven (110°C). Dissolve 0.7456 g of KCl in RODI water and bring to 1-L volume. Conductivity at 25°C is 1.412 mmhos cm^{-1}.

5.17.6 Procedure

1. Weigh 5.0 g of <2-mm, air-dry soil in a 30-mL (1-oz) condiment cup.
2. Add 10 mL of RO water to sample using a Repipet dispenser.
3. Swirl to mix, cap, and allow to stand overnight.
4. Standardize the conductivity bridge using RO water (blank) and 0.010 N KCl (1.41 mmhos cm^{-1}).
5. Read conductance of supernatant solution directly from the bridge.
6. Record conductance to 0.01 mmhos cm^{-1}.

5.17.7 Calculation

1. No calculations are required for this procedure.
2. Use the following relationship to estimate the total soluble cation or anion concentration (meq L^{-1}) in the soil.
3. EC (mmhos cm-1) x 10 = Cation or Anion (meq L^{-1})
4. Use the following relationship to estimate the total soluble cation or anion concentration (meq g^{-1} oven-dry soil) in the soil.

EC (mmhos cm^{-1}) x 20 = Cation (meq g^{-1} soil)

EC (mmhos cm^{-1}) x 20 = Anion (meq g^{-1} soil)

5.17.8 Reporting

Report prediction conductance to the nearest 0.01 mmhos cm^{-1} (dS m^{-1}).

5.17.9 References

U.S. Salinity Laboratory Staff. 1954. L.A. Richards (ed.) Diagnosis and improvement of saline and alkali soils. 160 p. USDA Handb. 60. U.S. Govt. Print. Office, Washington, DC.

5.18 Citrate Dithionite Extraction (Al, Fe, and Mn)

5.18.1 Summary of Method

A soil sample is mixed with sodium dithionite, sodium citrate, and reverse osmosis deionized (RODI) water, and shaken overnight. Solution is centrifuged, and a clear extract obtained. The CD extract is diluted with RODI water. The analytes are measured by an atomic absorption spectrophotometer (AAS). The data are automatically recorded by a computer and printer. The AAS converts absorption to analyte concentration.

5.18.2 Interferences

There are four types of interferences (matrix, spectral, chemical, and ionization) in the AA analyses of these elements. These interferences vary in importance, depending upon the particular analyte selected.

The redox potential of the extractant is dependent upon the pH of the extracting solution and the soil system. Sodium citrate complexes the reduced Fe and usually buffers the system to a pH of 6.5 to 7.3. Some soils may lower the pH, resulting in the precipitation of Fe sulfides.

Filtered extracts can yield different recoveries of Fe, Mn, and Al, relative to unfiltered extracts.

5.18.3 Precautions

Wear protective clothing (coats, aprons, sleeve guards, and gloves); eye protection (face shields, goggles, or safety glasses); and a breathing filter when handling dry sodium dithionite. Sodium dithionite may spontaneously ignite if allowed to become moist, even by atmospheric moisture. Keep dithionite in a fume hood.

Follow standard laboratory practices when handling compressed gases. Gas cylinders should be chained or bolted in an upright position. Acetylene gas is highly flammable. Avoid open flames and sparks. Standard laboratory equipment includes fire blankets and extinguishers for use when necessary. Follow the manufacturer's safety precautions when using the AAS.

5.18.4 Equipment/Materials

- Electronic balance, ±1.0-mg sensitivity
- Mechanical reciprocating shaker, 200 oscillations min-1, 1 ½ in strokes, Eberbach 6000, Eberbach Corp., Ann Arbor, MI
- Atomic absorption spectrophotometer (AAS), double-beam optical system, AAnalyst, 300, Perkin-Elmer Corp., Norwalk, CT, with computer and printer
- Autosampler, AS-90, Perkin-Elmer Corp., Norwalk, CT
- Peristaltic pump
- Single-stage regulators, acetylene and nitrous oxide
- Centrifuge, Centra, GP-8, Thermo IEC, Needham Heights, MA
- Digital diluter/dispenser, with syringes 10000 and 1000 µL, gas tight, MicroLab 500, Hamilton Co., Reno, NV
- Dispenser, 30 mL
- Test tubes, 15-mL, 16 mm x 100, for sample dilution and sample changer
- Containers, polypropylene
- Volumetrics, Class A, 100, 250, and 1000-mL
- Measuring scoop, handmade, 0.4 g calibrated
- Centrifuge tubes, 50-mL

5.18.5 Reagents

- Reverse osmosis deionized (RODI) water, ASTM Type I grade of reagent water
- Sodium dithionite ($Na_2S_2O_4$), purified powder
- Sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), crystal, reagent. Dissolve 336 g sodium citrate in approximately 1 L RODI water, followed by diluting to 2 L with RODI water. Final concentration is 0.57 M sodium citrate.
- Sulfuric acid (H_2SO_4), concentrated.
- Phosphoric acid (H_3PO_4), concentrated (85%). For Fe analysis, samples are diluted 1:50 prior to analysis. A diluting solution for Fe analysis (for a final concentration of 0.5% H_3PO_4 in samples) may be made by adding 6.12 mL of concentrated H_3PO_4 to 500 mL

volume of RODI water, diluting to 1000 mL and mixing thoroughly. (Note: 1:5 sample dilutions for Al and Mn are in RODI water.)

- Primary Stock Standard Solution (PSSS), high purity, 1000 mg L-1: Fe, Mn, and Al.
- Calibration standards for Fe (See next bullet): To each 100 mL volume of blank, calibration, and quality control (QC) standards, add 25 mL of the following matrix matching mixture. This mixture is made by combining 20 mL Na citrate extracting solution, 0.21 mL H_2SO_4, and 6 mL H_3PO_4 and diluting to 250 mL volume with RODI water. Invert to mix thoroughly. (Note: Matrix of standards is prepared to match a 1:50 dilution of samples. Also, H_2SO_4 substitutes for dithionite).
- Standard Fe calibration solutions (SFeCS) or working standards (25.0, 20.0, 15.0, 10.0, 5.0, 1.0, and 0.0 mg Fe L-1) and QC (12.5 mg L-1). Prepare fresh weekly. In seven 100-mL volumetric flasks add as follows:

    - 25.0 mg Fe L^{-1} = 2.5 mL PSSSFe
    - 20.0 mg Fe L^{-1} = 2.0 mL PSSSFe
    - 15.0 mg Fe L^{-1} = 1.5 mL PSSSFe
    - 10.0 mg Fe L^{-1} = 1.0 mL PSSSFe
    - 5.0 mg Fe L^{-1} = 0.5 mL PSSSFe
    - 1.0 mg Fe L^{-1} = 0.1 mL PSSSFe
    - 0.0 mg Fe L^{-1} = 0.0 mL PSSSFe (blank)
    - 12.5 m Fe L^{-1} = 1.25 mL PSSSFe (QC)

- Fill to volume with RODI water and invert to mix thoroughly. After dissolution, transfer solution to a plastic bottle.
- Calibration standards for Mn (See next bullet): To each 100 mL volume of blank, calibration, and quality control (QC) standards, add 25 mL of the following matrix matching mixture. This mixture is made by combining 200 mL Na citrate extracting solution, 2.1 mL H_2SO_4, and diluting to 250-mL volume with RODI water. Invert to mix thoroughly. (Note: Matrix of standards is prepared to match a 1:5 dilution of samples. Also, H_2SO_4 substitutes for dithionite).
- Standard Mn calibration solutions (SMnCS) or working standards (15.0, 10.0, 5.0, 2.5, 1.5, and 0.0 mg Mn L-1) and QC (6.5 mg L-1). Prepare fresh weekly. In six 100-mL volumetric flasks add as follows:

    - 15.0 mg Mn L^{-1} = 1.5 mL PSSSMn
    - 10.0 mg Mn L^{-1} = 1.0 mL PSSSMn
    - 5.0 mg Mn L^{-1} = 0.5 mL PSSSMn
    - 2.5 mg Mn L^{-1} = 0.25 mL PSSSMn
    - 1.5 mg Mn L^{-1} = 0.15 mL PSSSMn
    - 0.0mg Mn L^{-1} = 0.0 mL PSSSMn (blank)
    - 6.5 mg Mn L^{-1} = 0.65 mL PSSSMn (QC)

- Fill to volume with RODI water and invert to mix thoroughly. After dissolution, transfer solution to a plastic bottle.
- Calibration standards for Al (See next bullet): To each 100 mL volume of blank, calibration, and quality control (QC) standards, add 25 mL of the following matrix matching mixture. This mixture is made by combining 200 mL Na citrate extracting solution, 2.1 mL H2SO4, and then diluting to 250-mL volume with RODI water. Invert to mix thoroughly. (Note: Matrix of standards is prepared to match a 1:5 dilution of samples (same as with Mn). Also, H_2SO_4 substitutes for dithionite).
- Standard Al calibration solutions (SAlCS) or working standards (100.0, 80.0, 60.0, 40.0, 20.0, 10.0, and 0.0 mg Al L-1) and QC (50.0 mg L-1). Prepare fresh weekly. In seven 100-mL volumetric flasks add as follows:

    - 100.0 mg Al L^{-1} = 10.0 mL PSSSAl
    - 80.0 mg Al L^{-1} = 8.0 mL PSSSAl
    - 60.0 mg Al L^{-1} = 6.0 mL PSSSAl
    - 40.0 mg Al L^{-1} = 4.0 mL PSSSAl
    - 20.0 mg Al L^{-1} = 2.0 mL PSSSAl
    - 10.0 mg Al L^{-1} = 1.0 mL PSSSAl
    - 0.0 mg Al L^{-1} = 0.0 mL PSSSAl (blank)
    - 50.0 mg Al L^{-1} = 5.0 mL PSSSAl (QC)

- Fill to volume with RODI water and invert to mix thoroughly. After dissolution, transfer solution to a plastic bottle.
- Acetylene gas, purity 99.6%
- Nitrous oxide, USP
- Compressed air with water and oil traps

5.18.6 Procedure

Extraction of Al, Fe, and Mn

1. Weigh 0.75 g of <2-mm or fine-grind, air-dry soil sample to the nearest mg and place in an 50-mL centrifuge tube. If sample is moist, weigh enough soil to achieve ≈ 0.75 g of air-dry soil.

2. Add 0.4 g of sodium dithionite (use one calibrated scoop) and 25 mL of sodium citrate solution.

3. Cap tubes and shake briefly by hand to dislodge soil from tube bottom. Place tubes in rack.

4. Place rack in shaker and shake overnight (12 to 16 h) at 200 oscillations min^{-1} at room temperature (20°C ± 2°C).

5. Remove tubes from shaker and manually shake tubes to dislodge any soil from cap. Allow samples to sit overnight.

6. The following day, centrifuge at 4000 rpm for 15 min. The Fe, Mn, and Al are determined on the AAS from a clear aliquot of solution.

Dilution of Sample Extracts

No ionization suppressant is required as the Na in the extractant is present in sufficient quantity. For a 1:50 dilution of samples for Fe analysis, use the H_3PO_4 diluting solution (see Section 5.9.5, bullet #5). The dilution of Fe results in a final solution concentration of 0.5% H_3PO_4. Dilute 1 part CD sample extract with 49 parts of H_3PO_4 diluting solution (1:50 dilution).

7. A 1:5 dilution in RODI water is used for Al and Mn. Dilute 1 part CD sample extract with 4 parts RODI water.

8. Dispense the diluted sample solutions into test tubes that have been placed in the holders of the sample changer.

AAS Set-up and Operation

Refer to the manufacturer's manual for operation of the AAS. The following are only very general guidelines for instrument conditions for the various analytes.

Analyte	Conc. ($mg\ L^{-1}$)	Burner Head	Wavelength (nm)	Slit (mm)	Fuel/Oxidant (C_2H_2/Air)
Fe	25.0	10-cm parallel	248.8	0.2	3.0 / 15.7
Mn	15.0	10-cm parallel	279.8	0.2	3.0 / 15.7
Al	100.0	5-cm parallel	309.3	0.7	8.5 / 15.7

Typical read delay is 3 s, and integration time is 3 s but can vary depending on soil type. Three replicates are average for each sample.

9. Use the computer and printer to set instrument parameters and to collect and record instrument readings.

10. The instrument readings are programmed to display analyte concentration in $mg\ L^{-1}$ (ppm).

AAS Calibration and Analysis

Each element is analyzed during separate runs on the AAS. Use the calibration reagent blank and calibration standards to calibrate the AAS. Calibrations are linear with calculated intercept.

11. Use the QC after every 12th sample. It must pass within 15% to continue. If it fails, recalibrate and reread the QC. The QC is also read at the end of each run.

12. If samples are outside the calibration range, a serial dilution is performed. A 1:5 dilution of the sample using the calibration blank, followed by the typical dilution (1:5 dilution with RODI water for Al and Mn, and 1:50 dilution with the H_3PO_4 diluting solution for Fe). Maintain matrix match between standards and diluted samples by performing this extra dilution with calibration blank.

13. Record analyte readings to 0.01 unit.

5.18.7 Calculation

Convert analyte concentrations (mg L^{-1}) to percent in soil as follows:

$$\text{Soil Fe, Al, Mn (\%)} = (A \times B \times C \times R \times 100)/ (E \times 1000)$$

where:

A = Sample extract reading (mg L^{-1})
B = Extract Volume (L)
C = Dilution, required
R = Air-dry/oven-dry ratio (procedure 3D1) or field-moist/oven-dry ratio (procedure 3D2)
E = Sample weight (g)
100 = Conversion factor to 100-g basis
1000 = mg g^{-1}

5.18.8 Reporting

Report percent CD extractable Al, Fe, and Mn to the nearest 0.1 of a percent.

5.18.9 References

Wada, K. 1989. Allophane and imogolite. p. 1051-1087. In J.B. Dixon and S.B. Weed (eds.). Minerals in soil environment. 2nd ed. Soil Sci. Am. Book Series No. 1. ASA and SSSA, Madison, WI.

Soil Survey Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. USDA-NRCS Agric. Handb. 436. 2nd ed. U.S. Govt. Print. Office, Washington, DC.

5.19 Phosphorus (Olsen)

5.19.1 Summary of Method

A 1.0-g soil sample is shaken with 20 mL of Olsen sodium-bicarbonate extracting solution for 30 min. The sample is centrifuged until solution is free of soil mineral particles, and then filtered until clear extracts are obtained. Dilute 5-mL of sample extract with 5-mL of color reagent. The absorbance of the solution is read using a spectrophotometer at 882 nm. Data are reported as mg P kg^{-1} soil.

5.19.2 Interferences

The Mo blue methods, which are very sensitive for P, are based on the principle that in an acid molybdate solution containing orthophosphate ions, a phosphomolybdate complex forms that can be reduced by ascorbic acid, $SnCl_2$, and other reducing agents to a Mo color. The intensity of blue color varies with the P concentration but is also affected by other factors such as acidity, arsenates, silicates, and substances that influence the oxidation-reduction conditions of the system (Olsen and Sommers, 1982).

5.19.3 Precautions

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses). When preparing reagents, exercise special care. Many metal salts are extremely toxic and may be fatal if ingested. Thoroughly wash hands after handling these metal salts. Restrict the use of concentrated H_2SO_4 and HCl to a fume hood.

Use safety showers and eyewash stations to dilute spilled acids and bases. Use sodium bicarbonate and water to neutralize and dilute spilled acids.

5.19.4 Equipment/Materials

- Electronic balance, ±1.0-mg sensitivity
- Mechanical reciprocating shaker, 200 oscillations min-1, 1 1⁄2 in strokes, Eberbach 6000, Eberbach Corp., Ann Arbor, MI
- Centrifuge tube, 50 mL, polyethylene
- Funnel, 60° angle, long stem, 50-mm diameter
- Filter paper, Whatman 42, 150 mm
- Centrifuge, Centra, GP-8, Thermo IEC, Thermo IEC, Needham Heights, MA.
- Pipettes, electronic digital, 1000µL and 10 mL, with tips, 1000µL and 10 ml cups, plastic
- Dispenser, 30 mL or 10 mL
- Cuvettes, plastic, 4.5-mL, 1-cm light path, Daigger Scientific
- Spectrophotometer, UV-Visible, Dual-View, Varian, Cary 50 Conc, Varian Australia Pty Ltd.
- Computer, with Cary WinUV software, Varian Australia Pty Ltd., and printer

5.19.5 Reagents

- Sulfuric acid (H_2SO_4), concentrated, 36 N, trace pure grade
- Sulfuric acid (H_2SO_4), 4 M. To 250-mL volumetric, carefully add 56 mL concentrated H_2SO_4 to 150 mL RODI water. Allow to cool. Make to final volume with RODI water. Invert to thoroughly mix.
- NaOH, 1 M. Dissolve 4 g NaOH in 100 mL RODI water.
- Olsen Sodium Bicarbonate Extracting solution (0.5 M $NaHCO_3$). To 6-L container, dissolve 252 g $NaHCO_3$ in RODI water. Adjust the pH to 8.5 with 1M NaOH. Make to final volume. Mix thoroughly. Check pH every day.
- Ammonium molybdate, 4%. Dissolve 4 g of $[(NH_4)_6Mo_7O_{24}•4H_2O]$ in 100-mL volumetric with RODI water. Dilute to volume with RODI water. Store in the dark in the refrigerator.
- Potassium antimony – (III) oxide tartarate, 0.275%. Dissolve 0.275 g $[K(SbO)C_4H_4O_6•½H_2O]$ in 100 mL RODI water.
- Ascorbic acid, 1.75%. Dissolve 1.75 g ascorbic acid in 100 mL RODI water. Prepare fresh daily.
- Color developing reagent. To a 500-mL bottle, add 50 mL 4 M H_2SO_4, 15 mL 4% ammonium molybdate, 30 mL 1.75% ascorbic acid, 5 mL 0.275% potassium antimony – (III) oxide tartarate, and 200 mL RODI water. Mix well after each addition. Prepare fresh daily.

- Stock standard P solution (SSPS), 100.0 mg P L-1. In a 1-L volumetric flask, dissolve 0.4394 g primary standard grade anhydrous potassium dihydrogen phosphate (KH_2PO_4) that has been dried for 2 h at 110°C in about 800 mL extracting solution. Dilute to 1-L volume with extracting solution and invert to thoroughly mix. Store in polyethylene containers. Make fresh weekly. Store in a refrigerator.
- Working stock standard P solution (WSSPS), 4.0 mg P L-1. Pipet 10 mL of 100 mg P L-1 SSPS to 250-mL volumetric flask. Dilute to 250-mL volume with extracting solution and invert to thoroughly mix. Make fresh weekly. Store in the refrigerator.
- Standard P calibration solution (SPCS), or working standards, 2.0, 1.6, 1.2, 0.8, 0.4, and 0.0 mg P L-1. Make fresh weekly. Store in the refrigerator. Allow to equilibrate to room temperature before use. To six 50-mL volumetric flasks, add as follows:

    - 2.0 mg P L^{-1}= 25 mL WSSPS
    - 1.6 mg P L^{-1} = 20 mL WSSPS
    - 1.2 mg P L^{-1} = 15 mL WSSPS
    - 0.8 mg P L^{-1} = 10 mL WSSPS
    - 0.4 mg P L^{-1} = 5 mL WSSPS
    - 0.0 mg P L^{-1} = 0 mL WSSPS (blank)

- Dilute each SPCS to mark with extracting solution and invert to thoroughly mix.
- Quality Control Samples: 0.1mg P L-1 solution made from SSPS; blanks; and selected SPCS. In addition, SSL soil standard and WEPAL ISE's (Wageningen Evaluating Programmes for Analytical Laboratories, International Soil Exchange) from the Netherlands are routinely included in a batch for quality control.

5.19.6 Procedure

1. Weigh 1.0 g of <2mm or fine-grind, air-dry soil to the nearest mg into a 50-mL centrifuge tube. If sample is moist, weigh enough soil to achieve ≈ 1.0 g of air-dry soil.

2. Dispense 20.0 mL of extracting solution to tube.

3. Transfer the sample to the shaker. Shake for 30 min at 200 oscillations min^{-1} at room temperature (20°C± 2°C).

4. Remove the sample from the shaker. Centrifuge at 2000 rpm for 10 min, decant, filter, and collect extract in receiving cup. If extracts are not to be determined immediately after collection, then store samples at 4°C. Analyze samples within 72 h.

5. Use the pipette to transfer a 5-mL aliquot of the sample to a plastic cup. Also transfer a 5-mL aliquot of each SPCS to a plastic cup. Use a clean pipette tip for each sample and SPCS.

6. Dispense 5 mL of color developing reagent to sample aliquot and to each SPCS. Swirl to mix. Do not place sample cups close together as carbon dioxide is released and solution will bubble. The color reaction requires a minimum of 20 min before analyst records readings. Allowing 1 h for color development usually improves results. Color will remain stable for 24 h.

7. Transfer sample extract and SPCS to cuvettes.

8. Set the spectrophotometer to read at 882 nm. Autozero with calibration blank.

9. Calibrate the instrument using the SPCS. The data system will then associate the concentrations with the instrument responses for each SPCS. Rejection criteria for SPCS, if R2 <0.99.

10. Run samples using calibration curve. Sample concentration is calculated from the regression equation. Rejection criteria for batch, if blanks as samples > 0.01; if SPCS as samples > ± 20%; if SSL standard > ± 20% mean; and if ISE > (3 x MAD), where MAD = median of absolute deviations. Record results to the nearest 0.01 unit for the sample extract and each SPCS.

11. If samples are outside calibration range, dilute sample extracts with extracting solution and re-analyze.

5.19.7 Calculation

Convert the extract P (mg L^{-1}) to soil P (mg kg^{-1}) as follows:

$$\text{Soil P (mg kg}^{-1}\text{)} = [(A \times B \times C \times R \times 1000)/E]$$

where:

A = Sample extract reading (mg L^{-1})
B = Extract volume (L)
C = Dilution, if performed
R = Air-dry/oven-dry ratio (procedure 3D1) or field-moist/oven-dry ratio (procedure 3D2)
1000 = Conversion factor to kg-basis

E = Sample weight (g)

5.19.8 Reporting

Report data to the nearest 0.1 mg P kg^{-1} soil.

5.19.9 References

Buurman, P, B. van Lagen, and E.J. Velthorst. 1996. Manual for soil and water analysis. Backhuys Publ., Leiden, the Netherlands.

Burt, R., M..D. Mays, E.C. Benham, and M.A. Wilson. 2002. Phosphorus characterization and correlation with properties of selected benchmark soils of the United States. Commun. Soil Sci. Plant Anal. 33:117-141.

5.20 Phosphorus (Mehlich No. 3)

5.20.1 Summary of Method

A 2.5-g soil sample is shaken with 25 mL of Mehlich No. 3 extracting solution for 5 min. The sample is centrifuged until solution is free of soil mineral particles, and then filtered until clear extracts are obtained. Dilute 0.5-mL of sample extract with 13.5-mL of working solution. Absorbance of the solution is read using a spectrophotometer at 882 nm. Data are reported as mg P kg^{-1} soil.

5.20.2 Interferences

The Mo blue methods, which are very sensitive for P, are based on the principle that in an acid molybdate solution containing orthophosphate ions, a phosphomolybdate complex forms that can be reduced by ascorbic acid, $SnCl_2$, and other reducing agents to a Mo color, with ascorbic acid most commonly used by agricultural laboratories (Murphy and Riley, 1962). The intensity of blue color varies with the P concentration but is also affected by other factors such as acidity, arsenates, silicates, and substances that influence the oxidation-reduction conditions of the system (Olsen and Sommers, 1982).

5.20.3 Precautions

Wear protective clothing (coats, aprons, sleeve guards, and gloves) and eye protection (face shields, goggles, or safety glasses). When preparing reagents, exercise special care. Many metal salts are extremely toxic and may be fatal if ingested. Thoroughly wash hands after handling these metal salts. Restrict the use of concentrated H_2SO_4 and HCl to a fume hood. Use safety showers and eyewash stations to dilute spilled acids and bases. Use sodium bicarbonate and water to neutralize and dilute spilled acids.

5.20.4 Equipment/Materials

Electronic balance, ±1.0-mg sensitivity
Mechanical reciprocating shaker, 200 oscillations min-1, 1 1⁄2 in strokes, Eberbach 6000, Eberbach Corp., Ann Arbor, MI
Centrifuge tubes, 50-mL, polyethylene
Funnel, 60° angle, long stem, 50-mm diameter
Filter paper, Whatman 42, 150 mm
Centrifuge, Centra, GP-8, Thermo IEC, Needham Heights, MA
Pipettes, electronic digital, 1000 µL and 10 mL, with tips, 1000 µL and 10 mL
Dispenser, 30 mL or 10 mL
Cups, plastic
Cuvettes, plastic, 4.5-mL, 1-cm light path, Daigger Scientific
Spectrophotometer, UV-Visible, Dual-View, Varian, Cary 50 Conc, Varian Australia Pty Ltd.
Computer with Cary WinUV software, Varian Australia Pty Ltd., and printer

5.20.5 Reagents

- Reverse osmosis deionized (RODI) water, ASTM Type I grade of reagent water
- Sulfuric acid (H_2SO_4), concentrated, 36 N, trace pure grade
- Mehlich No. 3 Extracting solution (0.2 N CH_3COOH; 0.25 N NH_4NO_3; 0.015 N NH_4F; 0.13 N HNO_3; 0.001 M EDTA). Premixed Mehlich No. 3 Extractant, Special-20, Hawk Creek Laboratory, Rural Route 1, Box 686, Simpson Road, Glen Rock, PA, 17327.
- Sulfuric-tartarate-molybdate solution (STMS). Dissolve 100 g of ammonium molybdate tetrahydrate [$(NH_4)6Mo_7O_{24}•4H_2O$] in 500 mL of RODI water. Dissolve 2.425 g of antimony potassium tartarate (potassium antimony tartarate hemihydrate. [$K(SbO)C_4H_4O_6•1/2H_2O$] in the ammonium molybdate solution. Slowly and carefully add 1400 mL of concentrated

H_2SO_4 and mix well. Cool and dilute to 2 L with RODI water. Store in the dark in the refrigerator.

- Ascorbic acid solution. Dissolve 8.8 g of ascorbic acid in RODI water and dilute to 100- mL with RODI water. Make fresh daily.
- Working ascorbic acid molybdate solution (WAMS). Dilute 20 mL of STMS solution and 10 mL of the ascorbic acid solution with RODI water to make 1-L. Allow solution to come to room temperature before using. Prepare fresh daily.
- Working stock standard P solution (WSSPS), 100.0 mg P L-1. In a 1-L volumetric flask, dissolve 0.4394 g primary standard grade anhydrous potassium dihydrogen phosphate (KH_2PO_4) (dried for 2 h at 110°C) in about 800 mL extracting solution. Dilute to 1-L volume with extracting solution and invert to thoroughly mix. Store in polyethylene containers. Make fresh weekly. Store in a refrigerator.
- Standard P calibration solutions (SPCS) or working standards, 12.0, 10.0, 8.0, 4.0, 2.0, 1.0, 0.8, 0.4 and 0.0 mg P L-1. Prepare fresh weekly. Store in a refrigerator. Allow to equilibrate to room temperature before use. In nine 250-mL volumetric flasks add as follows:

    - 12.0 mg P L^{-1} = 30 mL WSSPS
    - 10.0 mg P L^{-1} = 25 mL WSSPS
    - 8.0 mg P L^{-1} = 20 mL WSSPS
    - 4.0 mg P L^{-1} = 10 mL WSSPS
    - 2.0 mg P L^{-1} = 5 mL WSSPS
    - 1.0 mg P L^{-1} = 2.5 mL WSSPS
    - 0.8 mg P L^{-1} = 2 mL WSSPS
    - 0.4 mg P L^{-1} = 1 mL WSSPS
    - 0.0 mg P L^{-1} = 0 mL WSSPS (blank)

- Dilute each SPCS to the mark with extracting solution and invert to thoroughly mix.
- Quality Control Samples: 0.1 mg P L -1 solution made from SSPS; blanks; and selected SPCS. In addition, SSL soil standard and WEPAL ISE's (Wageningen Evaluating Programmes for Analytical Laboratories, International Soil Exchange) from The Netherlands are routinely included in a batch for quality control.

5.20.6 Procedure

1. Weigh 2.5 g of <2-mm or fine-grind, air-dry soil to the nearest mg into a 50-mL centrifuge tube. If sample is moist, weigh enough soil to achieve ≈ 2.5 g of air-dry soil.

2. Dispense 25.0 mL of extracting solution to the tube.

3. Transfer the sample to the shaker. Shake for 5 min at 200 oscillations min-1 at room temperature (20°C± 2°C).

4. Remove the sample from the shaker. Centrifuge at 2000 rpm for 10 min, decant, filter, and collect extract in receiving cups. If extracts are not to be determined immediately after collection, then store samples at 4°C. Analyze samples within 72 h.

5. Use the pipette to transfer a 1 mL (or 0.5-mL) aliquot of the sample to a plastic cup. Also transfer a 1 mL (or 0.5-mL) aliquot of each SPCS to a plastic cup. Use a clean pipette tip for each sample and SPCS.

6. Dispense 27 mL (or 13.5 mL) of the WAMS to sample aliquot and to each SPCS. Swirl to mix. The color reaction requires a minimum of 20 min before analyst records readings. Allowing 1 h for color development usually improves results. Color will remain stable for 6 h.

7. Transfer sample extract and SPCS to cuvettes.

8. Set the spectrophotometer to read at 882 nm. Autozero with calibration blank.

9. Calibrate the instrument by using the SPCS. The data system will then associate the concentrations with the instrument responses for each SPCS. Rejection criteria for SPCS, if R2 <0.99.

10. Run samples using calibration curve. Sample concentration is calculated from the regression equation. Rejection criteria for batch, if blanks as samples > 0.01; if SPCS as samples > ± 20%; if SSL standard > ± 20% mean; and if ISE > (3 x MAD), where MAD = median of absolute deviations. Record results to the nearest 0.01 unit for the sample extract and each SPCS.

11. If samples are outside calibration range, dilute sample extracts with extracting solution and re-analyze.

5.20.7 Calculation

Convert extract P (mg L^{-1}) to soil P (mg kg^{-1}) as follows:

$$\text{Soil P (mg kg}^{-1}) = [(A \times B \times C \times R \times 1000)/E]$$

where:

A = Sample extract reading (mg L^{-1})

B = Extract volume (L)
C = Dilution, if performed

5.20.8 Reporting

Report data to the nearest 0.1mg P kg^{-1} soil.

5.20.9 References

Mehlich, A. 1984. Mehlich 3 soil text extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15:1409-1416.

Murphy, J., and J.R. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta. 27:31-36.

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. p. 403-430. In A.L. Page, R.H. Miller, and D.R. Keeney (eds.) Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Soil and Plant Analysis Council, Inc. 1999. Handbook on reference methods for soil analysis. Council on Soil Testing and Plant Analysis. CRC Press, Boca Raton, FL.

Tran, T.S., and R.R. Simard. 1993. Mehlich III-extractable elements. p. 43-50. In M.R. Carter (ed.) Soil sampling and methods of analysis. Can. Soc. Soil Sci. Lewis Publ., Boca Raton, FL.

5.21 Trace Elements (Ag, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, P, Pb, Sb, Se, Sn, Sr, Tl, V, W, and Zn)

5.21.1 Summary of Method

The approach of this digestion methodology is to maximize the extractable concentration of elements in digested soils while minimizing the matrix interferences such as found in digestion procedures that use HF acid. This method (4H1a1) follows EPA Method 3051A. A 500-mg <2-mm soil separate which has been air-dried and ground to < 200 mesh (75 μm) is weighed into a

100-ml Teflon (PFA) sample digestion vessel. To the vessel, 9.0 mL HNO_3 and 3.0 mL HCl are added. The vessel is inserted into a protection shield, covered and placed into a rotor with temperature control. Following microwave digestion the rotor and samples are allowed to cool, before quantitatively transferring the digestate into a 50-ml glass volumetric with high purity deionized reverse osmosis water (DIRO). The samples are then allowed to cool to room temperature before bringing to volume. Once brought to volume the samples are thoroughly mixed and allowed to settle overnight before decanting into 50ml Falcon tubes prior to analysis. The concentration of Ag, As, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, P, Pb, Sb, Se, Sn, V, W, and Zn are determined using an inductively coupled plasma mass spectrometer (ICP-MS).

5.21.2 Interferences

Organic constituents may contain metals and are difficult to digest if present in high concentrations. Certain elements are subject to volatile losses during digestion and transfer. Certain soil minerals (e.g., quartz, feldspars) are not soluble in HNO_3 + HCl.
Interferences are corrected or minimized by using an internal standard along with gas reaction and collision cell and technology. Also, careful selection of specific masses for data reporting is important. Interference corrections are made by ICP software. Samples and standards are matrix-matched to help reduce interferences.

5.21.3 Precautions

Wear protective clothing and eye protection. When preparing reagents, exercise special care. Restrict the use of concentrated acids to the fume hood. Wash hands thoroughly after handling reagents. Filling the digestion vessel to greater than 25 percent of the free volume or adding organic reagents or oxidizing agents to the vessel may result in explosion of the digestion microwave system.

5.21.4 Equipment/Materials

- Electronic balance, ±1.0-mg sensitivity
- Pipet(s) capable of delivering 3 and 9 mL, Omnifit Corp. manufacturers variable volume, (10-ml maximum) pipettes suitable for HNO3 and HCl delivery from 2.5 L bottles
- Volumetric flasks, class A glass, 50 mL
- Polypropylene bottles, 60 mL, with cap
- Electronic balance (±0.1 mg sensitivity)

- Microwave oven, CEM Mars 5, 14 position-HP500 Plus vessel and rotor (vessels composed of PFA, sleeves composed of advanced composite)
- Volumetrics, 500, 250, and 50-mL class A glass
- Containers, 500-mL, polypropylene, with screw caps
- Pipettes, electronic digital, 1000 µL and 10 mL, Rainin Instrument Co., Woburn, MA
- Inductively coupled plasma mass spectrometer (ICP-MS), Agilent 7500cx, Agilent Technologies Inc. Wilmington, Delaware
- Computer, with ICP-MS ChemStation software ver. B.03.07, Agilent Technologies Inc. Wilmington, Delaware
- Heat Exchanger, G1879B, Agilent Technolgies
- Compressed gasses, argon (minimum purity = 99.99%), hydrogen (minimum purity = 99.999%) and helium (minimum purity = 99.999%)
- Autosampler, ASX-500 Series, Agilent Technolgies Inc. Wilmington, De.
- Quartz torch, for use with HMI, Part No. G3270-80027
- Peristaltic pump (for automatic injection of internal standard)

5.21.5 Reagents

- Deinonized reverse osmosis (DIRO) water, ASTM Type I grade of reagent water
- Concentrated hydrochloric acid (HCl), 12 N, trace pure grade
- Concentrated nitric acid (HNO_3), 16 N, trace pure grade
- Primary standards: 1000 mg L-1, from High Purity Standards, Charleston, SC. Single and mixed element standards are manufactured in dilute HNO_3, HNO_3 + HF, or H_2O.

5.21.6 Procedure

Microwave Acid Digestion

1. About 500 mg of fine-earth (<2-mm) or a specific particle size separate ground to <200-mesh (75 µm) is weighed to the nearest 0.1 mg in a 100-mL digestion vessel.

Note: If sample is principally composed of organic materials (organic C > 15%), perform a preliminary digestion in the muffle furnace in an digestion crucible: 250°C for 15 min, 450°C for 15 min, followed by 550°C for 1 h.

2. Pipet 9.0 mL HNO_3 and 3.0 mL HCl into the sample and allow to completely wet. Add acids in the fume hood. Allow acids to react and vent in uncovered vessels for about 30 min.

3. Place covered vessels in protective sleeve, cover and place into rotor, attach pressure sensor to reference vessel, tighten all vessels to proper torque.

4. Place digestion rotor in the microwave oven and insert the temperature probe into the reference vessel. Attach the temperature and pressure sensor cables to the fittings in the microwave.

5. Microwave settings are as follows:
    - 1200 watts at 100% power for 5.5 min until 175°C
    - Hold at 175°C for 4.5 min
    - Cool for 5 min

6. After cooling, disconnect temperature probe and pressure sensor from microwave.

7. Remove rotor from oven, and place in fume hood.

8. Open each vessel carefully and then quantitatively transfer contents of vessel to a 50mL volumetric flask with DIRO water.

9. Cap flask and mix well by inverting. Allow samples to cool to room temperature. When cool, fill samples to volume with DIRO water and let settle overnight.

10. Decant contents into a labeled 60-mL polypropylene container.

11. Prepare working standards of a blank, reference soil sample from the SSL repository, NIST or other standard reference material and blank by the same digestion method. Run two of these standards and a blank with each set of 14 samples.

ICP-MS Calibration Standards, Set-Up, and Operation

12. Trace Method Stock C - Commercially prepared solution containing 10µg/mL Sb, As, Ba, Be, Cd, Cr, Co, Cu, Pb, Mo, Ni, Se, Sn, W, V, Zn, 1µg/mL Ag

13. Trace Method Stock A - Commercially prepared solution containing10µg/mL Mn, P, Sr

14. Mercury Stock Standard - Commercially prepared solution containing1µg/mL Hg

15. Standard Matrix - Place 500mL DIRO in a 1 L-volumetric flask, add 0.250mL 1000µg/mL Au and 18mL concentrated nitric acid. Fill to volume with DIRO and mix well.

16. TM7 - In a 1L flask add 300ml DIRO, 18mL concentrated nitric acid, 6mL concentrated hydrochloric acid, 0.250ml of 1000µg/mL Au, 1.0mL of Mercury Stock standard (1.13.6.15), fill to volume with DIRO and mix well.

17. TM6 - In a 1L flask add 300ml DIRO, 18mL concentrated nitric acid, 6mL concentrated hydrochloric acid, 0.250ml of 1000µg/mL Au, 0.5mL of Mercury Stock standard (1.13.6.15), fill to volume with DIRO and mix well.

18. TM5 - In a 500mL volumetric add 300mL DIRO and 50.0mL Trace Method Stock A (1.13.6.14), fill to volume with DIRO and mix well.

19. TM4 - In a 500mL volumetric add 300mL DIRO and 12.5mL Trace Method Stock A (1.13.6.14), fill to volume with DIRO and mix well.

20. TM3 - In a 500mL volumetric flask, add 300mL DIRO, 9mL of concentrated nitric acid, 0.125ml of 1000µg/mL Au and 0.5mL Trace Method Stock C (1.13.6.15). Fill to volume with DIRO, mix well.

21. TM2 - In a 500mL volumetric flask add 300mL of Standard Matrix (1.13.6.16) and 50mL TM3 (1.13.6.15). Fill to volume with Standard Matrix (1.13.6.16), mix well.

22. TM1 - In a 500mL volumetric add 300mL of Standard Matrix (1.13.6.16) and 50mL TM2 (1.13.6.22). Fill to volume with Standard Matrix (1.13.6.16), mix well.

23. TM0 - In a 500mL volumetric flask add 300mL DIRO, 9mL concentrated nitric acid, 3mL concentrated hydrochloric acid, 0.125ml of 1000µg/mL Au, fill to volume with DIRO and mix well.

24. Internal Standard (1µg/mL Li6 , Sc, Ge, Y, In, Tb, Bi) - In a 1L flask add 300mL DIRO, 18mL concentrated nitric acid, 6mL concentrated hydrochloric acid, .250ml of 1000µg/mL Au and 100mL of commercially prepared Internal Standard Mix, fill to volume with DIRO and mix well.

25. Rinse - In 2L flask add 300mL DIRO, 58mL concentrated nitric acid, fill to volume with DIRO and mix well.

26. Rinse #1 - In a 1L flask add 300mL DIRO, 29mL concentrated nitric acid, 1ml of 1000µg/mL Au, fill to volume with DIRO and mix well.

27. Rinse #2 - In a 1L flask add 300mL DIRO, 15mL concentrated nitric acid, 45mL concentrated hydrochloric acid and 1ml of 1000µg/mL Au, fill to volume with DIRO and mix well.

28. Sample diluent - In a 1L flask add 300mL DIRO, 0.277mL of 1000µg/mL Au, fill to volume with DIRO and mix well.

29. Standard concentrations in µg/mL for each element.

Element	TM0	TM1	TM2	TM3	TM4	TM5	TM6	TM7
Sb	0	0.1	1	10				
As	0	0.1	1	10				
Ba	0	0.1	1	10				
Be	0	0.1	1	10				
Cd	0	0.1	1	10				
Cr	0	0.1	1	10				
Co	0	0.1	1	10				
Cu	0	0.1	1	10				
Pb	0	0.1	1	10				
Mn	0				250	1000		
Hg	0						0.5	1
Mo	0	0.1	1	10				
Ni	0	0.1	1	10				
P	0	0.1	1	10	250	1000		
Se	0			10				
Ag	0	0.01	0.1	1				
Sr	0				250	1000		
Sn	0	0.1	1	10				
W	0	0.1	1	10				
V	0	0.1	1	10				
Zn	0	0.1	1	10				

30. Reporting m/z and tune step for each element analyzed.

Element	m/z	Tune 1 (H2)	Tune 2 (He)	Tune 3 (No Gas)
Sb	121			X
As	75		X	
Ba	137			X

Element	m/z	Tune 1 (H2)	Tune 2 (He)	Tune 3 (No Gas)
Be	9			X
Cd	111		X	
Cr	52		X	
Co	59		X	
Cu	63		X	
Pb	208			X
Mn	55		X	
Hg	202			X
Mo	98			X
Ni	60		X	
P	31		X	
Se	78	X		
Ag	107		X	
Sr	88			X
Sn	118	X	X	X
W	182		X	
V	51		X	
Zn	66		X	

31. Use the ICP-MS with a micromist nebulizer, quartz torch for micro flow nebulizer and quartz spray chamber to analyze samples. Internal standard is added via peristaltic pump using 0.19 mm id. pump tubing. Internal standard and samples or standards are mixed via coil prior to entering the nebulizer. Samples are diluted 1:10 or greater as necessary prior to analysis with sample diluent (1.13.6.29). Perform instrument checks (tune for sensitivity, resolution axis, P/A factor, internal standard RSD, torch alignment, EM tune) prior to analysis as discussed in operation manual of instrument. Check instrument gas pressures to ensure pressures are correct and of adequate supply.

32. Typical tune values for trace analysis method.

Tune 1 (H_2)

Plasma Parameters

RF Power	1550W
RF Matching	1.78V
Smpl Depth	8.5mm
Torch-H	0.4mm
Torch-V	0.2mm
Carrier Gas	0.90L/min
Makeup Gas	0.15L/min

Optional Gas	0.0
Nebulizer Pump	0.10rps
Sample Pump	0.0
S/C Temp	2degC

Ion Lenses

Extract 1	0.0V
Extract 2	-136.0V
Omega Bias-ce	-28V
Omega Lens-ce	0.0V
Cell Entrance	-40V
QP Focus	-8V
Cell Exit	-40V

Q-Pole Parameters

AMU Gain	130
AMU Offset	124
Axis Gain	0.9996
Axis Offset	0.04
QP Bias	-15.0V

Octapole Parameters

OctP RF	150V
OctP Bias	-18.0V

Reaction Cell

Reaction Mode	ON
H2 Gas	2.5mL/min
He Gas	0.0mL/min
Optional Gas	

Detector Parameters

Discriminator	8.0mV
Analog HV	1690V
Pulse HV	1260V

Tune 2 (He)

Plasma Parameters

RF Power	1550W
RF Matching	1.78V
Smpl Depth	8.5mm
Torch-H	0.4mm
Torch-V	0.2mm
Carrier Gas	0.90L/min
Makeup Gas	0.15L/min
Optional Gas	0.0
Nebulizer Pump	0.10rps
Sample Pump	0.0
S/C Temp	2degC

Ion Lenses

Extract 1	0.0V
Extract 2	-136.0V
Omega Bias-ce	-28V
Omega Lens-ce	0.0V
Cell Entrance	-40V
QP Focus	-10V
Cell Exit	-40V

Q-Pole Parameters

AMU Gain	130
AMU Offset	124
Axis Gain	0.9996
Axis Offset	0.04
QP Bias	-16.0V

Octapole Parameters

OctP RF	150V
OctP Bias	-18.0V

Reaction Cell

Reaction Mode	ON
H2 Gas	0.0mL/min
He Gas	6.0mL/min
Optional Gas	

Detector Parameters

Discriminator	8.0mV
Analog HV	1690V
Pulse HV	1260V

Tune 3 (No Gas)

Plasma Parameters

RF Power	1550W
RF Matching	1.78V
Smpl Depth	8.5mm
Torch-H	0.4mm
Torch-V	0.2mm
Carrier Gas	0.90L/min
Makeup Gas	0.15L/min
Optional Gas	0.0
Nebulizer Pump	0.10rps
Sample Pump	0.0
S/C Temp	2degC

Ion Lenses

Extract 1	0.0V
Extract 2	-136.0V
Omega Bias-ce	-28V
Omega Lens-ce	0.0V
Cell Entrance	-30V
QP Focus	3V

Cell Exit	-30V

Q-Pole Parameters

AMU Gain	130
AMU Offset	124
Axis Gain	0.9996
Axis Offset	0.04
QP Bias	-3.0V

Octapole Parameters

OctP RF	150V
OctP Bias	-6.0V

Reaction Cell

Reaction Mode	OFF
H2 Gas	
He Gas	
Optional Gas	

Detector Parameters

Discriminator	8.0mV
Analog HV	1690V
Pulse HV	1260V

33. Use the blank standard solution to dilute those samples with concentrations greater than the high standard. Rerun all elements and use only the data needed from the diluted analysis.

34. Establish detection limits using the blank standard solution. The instrumental detection limits are calculated by using 3 times the standard deviation of 10 readings of the blank. These values establish the lower detection limits for each element. Analyzed values lower than the detection limits are reported as "ND" or non-detected.

5.21.7 Calculation

The calculation of mg kg^{-1} of an element in the soil from µg L^{-1} in solution is as follows:

Analyte concentration in soil (mg kg^{-1}) = [A x B x C x R x 1000]/E x 1000

Where:

A = Sample extract reading (µg L^{-1})
B = Extract volume (L)
C = Dilution, if performed
R = Air-dry/oven-dry ratio (procedure 3D1)
1000 = Conversion factor in numerator to kg-basis
E = Sample weight (g)

1000 = Factor in denominator (µg mg^{-1})

5.21.8 Reporting

Analysis is generally done on one mass per element, if more than one mass is analyzed only the reporting mass is used for data reporting purposes. The particle-size fraction digested needs to be identified with each sample. Data are reported to the nearest 0.01 mg kg^{-1}.

5.21.9 References

Burt, R., M.A. Wilson, T.J. Keck, B.D. Dougherty, D.E. Strom, and J.A. Lindhal. 2002. Trace element speciation in selected smelter-contaminated soils in Anaconda and Deer Lodge Valley, Montana, USA. Adv. Environ. Res. 8:51-67.

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Jersak, J., R. Amundson, and G. Brimhall, Jr. 1997. Trace metal geochemistry in Spodosols of the Northeastern United States. J. Environ. Qual. 26:551-521.

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Wilcke, W., and W. Amelung. 1996. Small-scale heterogeneity of aluminum and heavy metals in aggregates along a climatic transect. Soil Sci. Soc. Am. Proc. 60:1490-1495.

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5.22 Bulk Density (Hammered Core, Field State)

Density is defined as mass per unit volume. Soil bulk density of a sample is the ratio of the mass of solids to the total or bulk volume. This total volume includes the volume of both solids and pore space. Bulk density is distinguished from particle density which is mass per unit volume of only the solid phase. Particle density excludes pore spaces between particles. As bulk density (Db) is usually reported for the <2-mm soil fabric, the mass and volume of rock fragments are subtracted from the total mass and volume.

Bulk density is highly dependent on soil conditions at the time of sampling. Changes in soil volume due to changes in water content will alter bulk density. Soil mass remains fixed, but the volume of soil may change as water content changes (Blake and Hartge, 1986). Bulk density, as a soil characteristic, is actually a function rather than a single value. Therefore, subscripts are added to the bulk density notation, Db, to designate the water state of the sample when the volume was measured. The SSL uses the bulk density notations of Db_f, Db_{33}, and Db_{od} for field-state, 33-kPa equilibration, and oven-dry, respectively.

Field-state (Db_f) is the bulk density of a soil sample at field-soil water content at time of sampling. The 33-kPa equilibration (Db_{33}) is the bulk density of a soil sample that has been desorbed to 33kPa (1/3 bar). The oven-dry (Db_{od}) is the bulk density of a soil sample that has been dried in an oven at 110°C.Bulk density also may be determined for field-moist soil cores of known volume. The bulk density of a weak or loose soil material for which the clod or core method is unsuitable may be determined by the compliant cavity method.

5.22.1 Summary of Method

A metal cylinder is pressed or driven into the soil. The cylinder is removed extracting a sample of known volume. The moist sample weight is recorded. The sample is then dried in an oven and weighed.

5.22.2 Interferences

During coring process, compaction of the sample is a common problem. Compression can be observed by comparing the soil elevation inside the cylinder with the original soil surface outside the cylinder. If compression is excessive, soil core may not be a valid sample for analysis. Rock fragments in the soil interfere with core collection. Dry or hard soils often shatter when hammering the cylinder into the soil. Pressing the cylinder into the soil reduces the risk of shattering the sample.

If soil cracks are present, select the sampling area so that crack space is representative of sample, if possible. If this is not possible, make measurements between the cracks and determine the aerial percentage of total cracks or of cracks in specimen.

5.22.3 Equipment

- Containers, air-tight, tared, with lids
- Electronic balance, ±0.01-g sensitivity
- Oven 110°C
- Sieve, No. 10 (2 mm-openings)
- Coring equipment. Sources described in Grossman and Reinsch (2002).

5.22.4 Procedure

1. Record the empty core weights (CW).

2. Prepare a flat surface, either horizontal or vertical, at the required depth in sampling pit.

3. Press or drive core sampler into soil. Use caution to prevent compaction. Remove core from the inner liner, trim protruding soil flush with ends of cylinder, and place in air-tight container for transport to laboratory. If soil is too loose to remain in the liner, use core sampler without the inner liner and deposit only the soil sample in air-tight container. Moisture can also be pushed directly into a prepared face. For fibrous organic materials, trim sample to fit snugly into a can.

4. Dry core in an oven at 110°C until weight is constant. Record oven-dry weight (ODW).

5. Measure and record cylinder volume (CV).

6. If sample contains rock fragments, wet sieve sample through a 2-mm sieve. Dry and weigh the rock fragments that are retained on sieve. Record weight of rock fragments (RF).

7. Determine density of rock fragments (PD).

5.22.5 Calculations

$$Db = (ODW - RF – CW)/[CV - (RF/PD)]$$

where:

Db = Bulk density of < 2-mm fabric at sampled, field water state (g cm-3)
ODW = Oven-dry weight
RF = Weight of rock fragments
CW = Empty core weight
CV = Core volume
PD = Density of rock fragments

5.22.6 Report

Bulk density is reported as g cc-1 to the nearest 0.01 g cm^{-3}.

5.22.7 References

Grossman, R.B. and T.G. Reinsch. 2002. Bulk density and linear extensibility. p. 201-228. In J.H. Dane and G.C. Topp (eds.) Methods of soil analysis, Part 4. Physical methods. Soil Sci. Am. Book Series No. 5. ASA and SSSA, Madison, WI.

5.23 Bulk Density (Saran Clod, Field State)

Density is defined as mass per unit volume. Soil bulk density of a sample is the ratio of the mass of solids to the total or bulk volume. This total volume includes the volume of both solids and pore space. Bulk density is distinguished from particle density which is mass per unit volume of only the solid phase. Particle density excludes pore spaces between particles. As bulk density (Db) is usually reported for the <2-mm soil fabric, the mass and volume of rock fragments are subtracted from the total mass and volume.

Bulk density is highly dependent on soil conditions at the time of sampling. Changes in soil volume due to changes in water content will alter bulk density. Soil mass remains fixed, but the volume of soil may change as water content changes (Blake and Hartge, 1986). Bulk density, as a soil characteristic, is actually a function rather than a single value. Therefore, subscripts are added to the bulk density notation, Db, to designate the water state of the sample when the volume was measured. The SSL uses the bulk density notations of Db_f, Db_{33}, and Db_{od} for field-state, 33-kPa equilibration, and oven-dry, respectively.

Field-state (Db_f) is the bulk density of a soil sample at field-soil water content at time of sampling. The 33-kPa equilibration (Db_{33}) is the bulk density of a soil sample that has been desorbed to 33kPa (1/3 bar). The oven-dry (Db_{od}) is the bulk density of a soil sample that has been dried in an oven at 110°C. Bulk density also may be determined for field-moist soil cores of known volume (procedure 3B6a). The bulk density of a weak or loose soil material for which the clod or core method is unsuitable may be determined by the compliant cavity method (procedure 3B3a).

5.23.1 Summary of Method

Field-occurring fabric (clods) is collected from the face of an excavation. One coat of plastic lacquer is applied in the field. Additional coats of plastic lacquer are applied in the laboratory. In its field-water state or after equilibration, the clod is weighed in air to measure its mass and in water to measure its volume. After the clod is dried in oven at 110°C, its mass and volume are determined again. A correction is made for the mass and volume of rock fragments and plastic coatings (Brasher et al., 1966; Blake and Hartge, 1986; Grossman and Reinsch, 2002).

5.23.2 Interferences

Errors are caused by non-representative samples. Only field-occurring fabric (clods) should be sampled. The whole soil bulk density may be overestimated because sampled clods frequently exclude the crack space between clods (Grossman and Reinsch, 2002).

The penetration of plastic lacquer into the voids of sandy or organic soils interferes with the corrections for mass and volume of the plastic coat and with the accuracy of water content determinations. Penetration can be reduced by spraying water on the clod followed by immediately dipping the clod in the plastic lacquer.

Loss of soil during the procedure will void the analyses because all calculations are based on the oven-dry soil mass. Holes in the plastic coating, which are detected by escaping air bubbles

from submerged clod, introduce errors in volume measurement. An inadequate evaporation of the plastic solvent results in overestimation of the soil mass. A drying time of 1 h is usually sufficient time for evaporation of solvent. However, clods with high organic matter content may need to dry longer.

Bulk density is reported for <2-mm soil fabric. Correction for rock fragments >2-mm requires either knowledge or assumption of the rock fragment density. Estimate or measurement errors of rock fragment density will affect the accuracy of the value for soil bulk density. Rock fragments may contain water which complicates the application to actual water-holding capacity.

5.23.3 Precautions

Methyl ethyl ketone (MEK) is extremely flammable. A type B fire extinguisher should be in close proximity in the laboratory. No open flames or nearby operation of electrical equipment are permitted while using MEK. The MEK vapor is classified as a sensory irritant. The 8-h time-weighted average (TWA) exposure limit is 200 ppm, and the short-term exposure limit (STEL) is 300 ppm (Occupational Safety and Health Administration, 1989). Avoid physical contact. Use with adequate ventilation. In closed buildings, use a fume hood. Keep in tightly closed containers. Use safety glasses, proper gloves, and a lab coat. Wash hands immediately after handling MEK. Additional information on the safe handling of MEK is available in Chemical Safety Data Sheet SD-83, Manufacturing Chemists' Association, Inc., 1825 Connecticut Ave. NW, Washington, DC.

Saran F-310 resin will decompose rapidly at temperatures >200°C releasing hydrogen chloride gas. Avoid contact with Fe, Zn, Cu, and Al in solution. Avoid all contact with strong bases.

5.23.4 Equipment

- Electronic balance, ±0.01-g sensitivity
- Rigid shipping containers. The SSL uses a corrugated box with compartments.
- Plastic bags, 1 mL, 127 x 89 x 330 mm
- Wire. The SSL uses a 28-awg coated copper wire.
- Hairnets
- Stock tags, 25.4-mm (1-in) diameter paper tag, with metal rim
- Hook assembly for weighing below balance
- Plexiglass water tank mounted on a fulcrum and lever to elevate tank
- Oven, 110°C
- Sieve, No. 10 (2 mm openings)

- Rope, 3 m
- Clothespins
- Silt loam soil
- Hot plate
- Spray bottle
- Liquid vapor trap. The SSL constructs a tin enclosure over hot plate with a chimney and duct to transfer vapor to water stream.

5.23.5 Reagents

- Methyl ethyl ketone (MEK), practical (2-butanone)
- Water
- Alcohol
- Liquid detergent. The SSL uses Liqui Nox.
- Dow Saran F-310 Resin, available from Dow Chemical Company.
- Plastic lacquer. Prepare plastic lacquer with resin to solvent ratios of 1:4 and 1:7 on a weight basis. Fill a 3.8-L (1-gal) metal paint can with 2700 ± 200 mL of solvent. Fill to the bottom of handle rivet. Add 540 or 305 g of resin to make 1:4 or 1:7 plastic lacquer, respectively. For the initial field and laboratory coatings, use the 1:4 plastic lacquer. Use 1:7 plastic lacquer for the last two laboratory coats. The 1:7 plastic lacquer is used to conserve the resin and to reduce cost. In the field, mix solvent with a wooden stick. In the laboratory, stir solvent with a non-sparking, high speed stirrer while slowly adding resin. Stir plastic lacquer for 15 min at 25°C. Store plastic lacquer in covered plastic or steel containers. Acetone may be substituted for MEK.

5.23.6 Procedure

Laboratory

1. Prepare a round stock tag with sample identification number. Cut the copper wire and loop around the clod. Record weight (TAG) of tag and wire. Loop fine copper wire around clod, leaving a tail to which round stock tag is attached. Record weight of clod (CC1).

2. Dip clod in 1:4 plastic lacquer. Wait 7 min and then dip clod in 1:7 plastic lacquer. Wait 12 min and then dip clod in 1:7 plastic lacquer. Wait 55 min and then reweigh clod. If clod has adsorbed >3% plastic by clod weight or smells excessively of solvent, allow longer drying time, then reweigh clod and record weight (CC2).

3. The clod should be waterproof and ready for volume measurement by water displacement. Suspend the clod below the balance, submerge in water, and record weight (WMCW).

4. Dry clod in an oven at 110°C until weight is constant. Weigh oven-dry clod in air (WODC) and in water (WODCW) and record weights.

5. If clod contains >5% rock fragments by weight, remove them from clod. Place clod in a beaker and place on hot plate. Cover hot plate with a liquid vapor trap. Use a fume hood. Heat clod on hot plate in excess of 200°C for 3 to 4 h. The plastic coating disintegrates at temperatures above 200°C. After heating, clod should appear black and charred. Remove clod from hot plate, lightly coat with liquid detergent, and add hot water.

6. Wet sieve the cool soil through a 2-mm, square-hole sieve. Dry and record weight (RF) of rock fragments that are retained on the sieve. Determine rock fragment density by weighing them in air to obtain their mass and in water to obtain their volume. If rock fragments are porous and have a density similar to soil sample, do not correct clod mass and volume measurement for rock fragments. Correct for rock fragments if these fragments can withstand breakdown when dry soil is placed abruptly in water or calgon.

7. Correct bulk density for weight and volume of plastic coating. The coating has an air-dry density of ≈ 1.3 g cm-3. The coating loses 10 to 20% of its air-dry weight when dried in oven at 110°C.

5.23.7 Calculation

Db_f = [WODC - RF - ODPC – TAG]/{[(CC2 - WMCW)/WD] - (RF/PD) - (MPC/1.3)}

where:

Dbf = Bulk density in g cc-1 of <2 mm fabric at field-sampled water state
WODC = Weight of oven-dry coated clod
RF = Weight of rock fragments
ODPC = MPC x 0.85, weight of oven-dry plastic coat
TAG = Weight of tag and wire
CC2 = Weight of tag and wire
WMCW = Weight of coated clod in water before oven drying
WD = Water density
PD = Density of rock fragments

$$MPC = [(CC2 - CC1) + FCE] \times RV$$

where:

MPC = Weight of plastic coat before oven-drying
CC1 = Weight of clod before three laboratory plastic coats
RV = Percent estimate of remaining clod volume after cutting to obtain flat surface (≈ 80%)

$$FCE = 1.5 \times [(CC2 - CC1)/3]$$

where:

FCE = Estimate of field-applied plastic coat

$$Db_{od} = [WODC – RF – ODPC - TAG]/\{[(WODC – WODCW)/WD] - (RF/PD) - (MPC/1.3)\}$$

where:

Db_{od} = Bulk density in g cm-3 <2 mm fabric at oven dryness
WODCW = Weight of oven-dry coated clod in water

$$W_f = \{[(CC2 – MPC) – (WODC – ODPC)]/[WODC - RF - ODPC – TAG]\} \times 100$$

where:

Wf = Percent water weight in sampled clod

5.23.8 Reporting

Bulk density is reported to the nearest 0.01g cm^{-3}.

5.23.9 References

Blake, G.R. and K.H. Hartge. 1986. Bulk density. p. 363-382. In A. Klute (ed.) Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Brasher, B.R., D.P. Franzmeier, V.T. Volassis, and S.E. Davidson. 1966. Use of saran resin to coat natural soil clods for bulk density and water retention measurements. Soil Sci. 101:108.

Grossman, R.B., and T.G. Reinsch. 2002. Bulk density and linear extensibility. p. 202-228. In J.H. Dane and G.C. Topp (eds.) Methods of soil analysis, Part 4. Physical methods. Soil Sci. Am. Book Series No. 5. ASA and SSSA, Madison, WI.

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5.24 Bulk Density (Oven-Dry, clods and cores)

5.24.1 Summary of Method

Field-occurring fabric (clods or cores) is collected from the face of an excavation. One coat of plastic lacquer is applied in the field. Additional coats of plastic lacquer are applied in the laboratory. The clod is dried in an oven at 110°C and then weighed in air to measure its mass and in water to measure its volume. A correction is made for the mass and volume of rock fragments and for plastic coatings (Brasher et al., 1966; Blake and Hartge, 1986).

5.24.2 Interferences

Errors are caused by non-representative samples. Only field-occurring fabric (clods) should be sampled. The whole soil bulk density may be overestimated because sampled clods frequently exclude the crack space between clods (Grossman and Reinsch, 2002).

The penetration of plastic lacquer into the voids of sandy or organic soils interferes with the corrections for mass and volume of the plastic coat and with the accuracy of water content determinations. Penetration can be reduced by spraying water on the clod followed by immediately dipping the clod in the plastic lacquer. Dipping should be done as quickly as possible to reduce penetration of plastic.

Loss of soil during the procedure will void the analyses because all calculations are based on the oven-dry soil mass. Holes in the plastic coating, which are detected by escaping air bubbles from submerged clod, introduce errors in volume measurement. An inadequate evaporation of plastic solvent results in overestimation of the soil mass. A drying time of 1 h is usually sufficient time for evaporation of solvent. However, clods with high organic matter content may need to dry longer.

Bulk density is reported for <2-mm soil fabric. Correction for rock fragments >2-mm requires either knowledge or assumption of the rock fragment density. Estimate or measurement errors of rock fragment density will affect the accuracy of the value for soil bulk density. Rock fragments may contain water, which complicates the application to actual water-holding capacity.

5.24.3 Safety

Methyl ethyl ketone (MEK) is extremely flammable. A type B fire extinguisher should be in close proximity in the laboratory. No open flames or nearby operation of electrical equipment are permitted while using MEK. The MEK vapor is classified as a sensory irritant. The 8-h time weighted average (TWA) exposure limit is 200 ppm, and the short-term exposure limit (STEL) is 300 ppm (Occupational Safety and Health Administration, 1989). Avoid physical contact. Use with adequate ventilation. In closed buildings, use a fume hood. Keep in tightly closed containers. Avoid physical contact. Use safety glasses, proper gloves, and a lab coat. Wash hands immediately after handling MEK. Additional information on the safe handling of MEK is available in Chemical Safety Data Sheet SD-83, Manufacturing Chemists' Association, Inc., 1825 Connecticut Ave. NW, Washington, DC.

Saran F-310 resin will decompose rapidly at temperatures >200°C releasing hydrogen chloride gas. Avoid contact with Fe, Zn, Cu, and Al in solution. Avoid all contact with strong bases.

5.24.4 Equipment

- Electronic balance, ±0.01-g sensitivity
- Rigid shipping containers. The SSL uses a corrugated box with compartments.
- Plastic bags, 1 mil, 127 x 89 x 330 mm
- Wire. The SSL uses a 28-awg coated copper wire.
- Hairnets
- Stock tags, 25.4-mm (1-in) diameter paper tag with metal rim

- Hook assembly for weighing below balance
- Plexiglass water tank mounted on a fulcrum and lever
- Oven, 110°C
- Sieve, no. 10 (2 mm openings)
- Rope, 3 m
- Clothespins
- Hot plate
- Spray bottle
- Liquid vapor trap. The SSL constructs a tin enclosure over hot plate with a chimney and duct to transfer vapor to water stream.
- Tension table. The SSL constructs a tension table by placing porous firebricks, covered with reinforced paper towels, in a tub of water.

5.24.5 Reagents

- Methyl ethyl ketone (MEK), practical (2-butanone).
- Water.
- Liquid detergent. The SSL uses Liqui Nox.
- Dow Saran F-310 Resin, available from Dow Chemical Company.
- Plastic lacquer. Prepare plastic lacquer with resin to solvent ratios of 1:4 and 1:7 on a weight basis. Fill a 3.8-L (1-gal) metal paint can with 2700 ±200 mL of solvent. Fill to the bottom of handle rivet. Add 540 or 305 g of resin to make 1:4 or 1:7 plastic lacquer, respectively. For the initial field and laboratory coatings, use the 1:4 plastic lacquer. Use 1:7 plastic lacquer for the last two laboratory coats. The 1:7 plastic lacquer is used to conserve the resin and to reduce cost. In the field, mix solvent with a wooden stick. In the laboratory, stir solvent with a non-sparking, high-speed stirrer while slowly adding resin. Stir plastic lacquer for 15 min at 25°C. Store plastic lacquer in covered plastic or steel containers. Acetone may be substituted for MEK.

5.24.6 Procedure

1. Prepare a round stock tag with sample identification number. Cut the copper wire and loop around the clod. Record weight (TAG) of tag and wire. Loop fine copper wire around clod, leaving a tail to which round stock tag is attached. Record weight of clod (CC1).

2. Dip clod in 1:4 plastic lacquer. Wait 7 min and then dip clod in 1:7 plastic lacquer. Wait 12 min and then dip clod in 1:7 plastic lacquer. Wait 55 min and then reweigh clod. If clod has

adsorbed >3% plastic by clod weight or smells excessively of solvent, allow longer drying time, then reweigh clod and record weight (CC2).

3. Dry clod in an oven at 110°C until weight is constant. Weigh oven-dry clod in air (WODC) and in water (WODCW) and record weights.

4. If clod contains >5% rock fragments by weight, remove them from clod. Place clod in a beaker and place on hot plate. Cover hot plate with a liquid vapor trap. Use a fume hood. Heat clod on hot plate in excess of 200°C for 3 to 4 h. The plastic coating disintegrates at temperatures above 200°C. After heating, clod should appear black and charred. Remove clod from hot plate, lightly coat with liquid detergent, and add hot water.

5. Wet sieve the cool soil through a 2-mm, square- hole sieve. Dry and record weight (RF) of rock fragments that are retained on the sieve. Determine rock fragment density by weighing them in air to obtain their mass and in water to obtain their volume. If rock fragments are porous and have a density similar to soil sample, do not correct clod mass and volume measurement for rock fragments. If rock fragments are porous and have a density similar to soil sample, do not correct clod mass and volume measurement for rock fragments. Correct for rock fragments if these fragments can withstand breakdown when dry soil is placed abruptly in water or calgon.

6. Correct bulk density for weight and volume of plastic coating. The coating has an air-dry density of ≈ 1.3 g cm-3. The coating loses 10 to 20% of its air-dry weight when dried in oven at 110°C.

5.24.7 Calculations

$$Db_{od} = [WODC- RF-ODPC – TAG]/\{[(WODC – WODCW)/WD] - (RF/PD) - (MPC/1.3)\}$$

where:

Db_{od}	= Bulk density in g cm-3 of <2 mm, oven-dry fabric
WODC	= Weight of oven-dry coated clod
RF	= Weight of rock fragments
TAG	= Weight of tag and wire
ODPC	= MPC1 x 0.85, weight of oven-dry plastic coat
WD	= Water density
PD	= Density of rock fragments
WODCW	= Weight of oven-dry coated clod in water

$$MPC = [(CC2 - CC1) + FCE] \times RV$$

where:

MPC = Weight of plastic coat before oven-drying
CC2 = Weight of clod after three laboratory plastic coats
CC1 = Weight of clod before three laboratory plastic coats
RV = Percent estimate of remaining clod volume after cutting to obtain flat surface (≈ 80%)

$$FCE = 1.5 \times [(CC2 - CC1)/3]$$

where:

FCE = Estimate of field-applied plastic coat

5.24.8 Report

Bulk density is reported to the nearest 0.01g cm^{-3}.

5.24.9 References

Blake, G.R., and K.H. Hartge. 1986. Bulk density. p. 363-382. In A. Klute (ed.) Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Brasher, B.R., D.P. Franzmeier, V.T. Volassis, and S.E. Davidson. 1966. Use of saran resin to coat natural soil clods for bulk density and water retention measurements. Soil Sci. 101:108.

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Occupational Safety and Health Administration. 1989. Air contaminants; final rule. Federal Register. 29 CFR Part 1910. Vol. 54. No. 12. p. 2452-2453. U.S. Govt. Print. Office, Washington, DC.

5.25 Bulk Density (33-kPA Desorption, clods only)

5.25.1 Summary of Method

Field-occurring fabric (clods only) are collected from the face of an excavation. One coat of plastic lacquer is applied in the field. Additional coats of plastic lacquer are applied in the laboratory. The clod is desorbed to 33 kPa. After equilibration, the clod is weighed in air to measure its mass and in water to measure its volume. After the clod is dried in the oven at 110°C, its mass and volume are determined again. A correction is made for the mass and volume of rock fragments and for plastic coatings (Brasher et al., 1966; Blake and Hartge, 1986).

5.25.2 Interferences

Errors are caused by non-representative samples. Only field-occurring fabric (clods) should be sampled. The whole soil bulk density may be overestimated because sampled clods frequently exclude the crack space between clods (Grossman and Reinsch, 2002).

The penetration of plastic lacquer into the voids of sandy and organic soils interferes with the corrections for mass and volume of the plastic coat and with the accuracy of water content determinations. Penetration can be reduced by spraying water on the clod followed by immediately dipping the clod in the plastic lacquer. Dipping should be done as quickly as possible to reduce penetration of plastic.

Loss of soil during the procedure will void the analyses because all calculations are based on the oven-dry soil mass. Holes in the plastic coating, which are detected by escaping air bubbles from submerged clod, introduce errors in volume measurement. An inadequate evaporation of plastic solvent results in overestimation of the soil mass. A drying time of 1 h is usually sufficient time for evaporation of solvent. However, clods with high organic matter content may need to dry longer.

Clods placed in an unsealed plastic bag can lose moisture during storage prior to analysis. If clods irreversibly dry below 33-kPa-water content, then Db1/3 values for 33 kPa will be erroneous. Completely seal the plastic storage bag to prevent drying.

Bulk density is reported for <2-mm soil fabric. Correction for rock fragments >2-mm requires either knowledge or assumption of the rock fragment density. Estimate or measurement errors of rock fragment density will affect the accuracy of the value for soil bulk density. Rock

fragments may contain water, which complicates the application to actual water-holding capacity.

5.25.3 Safety

Methyl ethyl ketone (MEK) is extremely flammable. A type B fire extinguisher should be in close proximity in the laboratory. No open flames or nearby operation of electrical equipment are permitted while using MEK. The MEK vapor is classified as a sensory irritant. The 8-h time weighted average (TWA) exposure limit is 200 ppm, and the short-term exposure limit (STEL) is 300 ppm (Occupational Safety and Health Administration, 1989). Avoid physical contact. Use with adequate ventilation. In closed buildings, use a fume hood. Keep in tightly closed containers. Use safety glasses, proper gloves, and a lab coat. Wash hands immediately after handling MEK. Additional information on the safe handling of MEK is available in Chemical Safety Data Sheet SD-83, Manufacturing Chemists' Association, Inc., 1825 Connecticut Ave. NW, Washington, DC.

Saran F-310 resin will decompose rapidly at temperatures >200°C releasing hydrogen chloride gas. Avoid contact with Fe, Zn, Cu, and Al in solution. Avoid all contact with strong bases.

5.25.4 Equipment

- Electronic balance, ±0.01-g sensitivity
- Pressure plate extractor with porous ceramic plate.
- Air pressure, 33-kPa
- Rigid shipping containers. The SSL uses a corrugated box with compartments.
- Plastic bags, 1 mil, 127 x 89 x 330 mm
- Wire. The SSL uses a 28-awg coated copper wire.
- Hairnets
- Stock tags, 25.4-mm (1-in) diameter paper tag, with metal rim
- Hook assembly for weighing below balance
- Plexiglass water tank mounted on a fulcrum and lever to elevate tank
- Oven, 110°C
- Sieve, No. 10 (2 mm openings)
- Rope, 3 m
- Clothespins
- Knife
- Tile cut off saw with diamond blade
- Silt loam soil

- Hot plate
- Desiccator with ceramic plate
- Vacuum, 80 kPa (0.8 bar)
- Metal probe
- Spray bottle
- Liquid vapor trap. The SSL constructs a tin enclosure over hot plate with a chimney and duct to transfer vapor to water stream.
- Reinforced paper towels or cheesecloth
- Tension table. The SSL constructs a tension table by placing porous firebricks, covered with reinforced paper towels, in a tub of water.

5.25.5 Reagents

- Methyl ethyl ketone (MEK), practical (2-butanone)
- Water
- Alcohol
- Liquid detergent. The SSL uses Liqui Nox.
- Dow Saran F-310 Resin, available from Dow Chemical Company.
- Plastic lacquer. Prepare plastic lacquer with resin to solvent ratios of 1:4 and 1:7 on a weight basis. Fill a 3.8-L (1-gal) metal paint can with 2700 ±200 mL of solvent. Fill to the bottom of handle rivet. Add 540 or 305 g of resin to make 1:4 or 1:7 plastic lacquer, respectively. For the initial field and laboratory coatings, use the 1:4 plastic lacquer. Use 1:7 plastic lacquer for the last two laboratory coats. The 1:7 plastic lacquer is used to conserve the resin and to reduce cost. In the field, mix solvent with a wooden stick. In the laboratory, stir solvent with a non-sparking, high-speed stirrer while slowly adding resin. Stir plastic lacquer for 15 min at 25°C. Store plastic lacquer in covered plastic or steel containers. Acetone may be substituted for MEK.

5.25.6 Procedure

1. Prepare a round stock tag with sample identification number. Cut the copper wire and loop around the clod (Fig. 1). Record weight (TAG) of tag and wire. Loop fine copper wire around clod, leaving a tail to which round stock tag is attached. Record weight of clod (CC1).

2. Dip clod in 1:4 plastic lacquer. Wait 7 min and then dip clod in 1:7 plastic lacquer. Wait 12 min and then dip clod in 1:7 plastic lacquer. Wait 55 min and then reweigh clod. If clod has

adsorbed >3% plastic by clod weight or smells excessively of solvent, allow longer drying time, then reweigh clod and record weight (CC2).

3. With a diamond saw, cut a flat surface on the clod. Place cut clod surface on a tension table, maintained at 5 cm tension (Fig.2). Periodically check clod to determine if it has reached equilibrium by inserting metal probe, touching, or by weight comparison. When clod has reached equilibrium, remove clod and record weight (WSC).

4. If cut clod does not adsorb water, place clod in a desiccator on a water-covered plate with a 0-cm tension. Submerge only the surface of clod in the water. Add a few mL of alcohol. Use in-house vacuum and apply suction until clod has equilibrated at saturation. Remove clod and record weight (WSC).

5. Place clod in a pressure plate extractor. To provide good contact between clod and ceramic plate, cover ceramic plate with a 5 mm layer of silt loam soil and saturate with water. Place a sheet of reinforced paper towel or cheesecloth over the silt loam soil. Place surface of cut clod on paper towel. Close container and secure lid. Apply gauged air pressure of 33 kPa. When water ceases to discharge from outflow tube, clod is at equilibrium. Extraction usually takes 3 to 4 wk. Remove clod and record weight (WMC). Compare WMC to WSC. If WMC > WSC, equilibrate clod on tension table and repeat desorption process.

6. Dip clod in the 1:4 plastic lacquer. Wait 7 min and then dip clod in 1:7 plastic lacquer. Wait 12 min and then dip clod in 1:7 plastic lacquer. Wait 12 min and dip clod in 1:7 plastic lacquer. After 55 min, reweigh clod and record weight (CC3). If clod has adsorbed >3% plastic by weight or smells excessively of solvent, allow longer drying time, then reweigh clod.

7. The clod should be waterproof and ready for volume measurement by water displacement. Suspend clod below the balance, submerge in water, and record weight (WMCW).

8. Dry clod in an oven at 110°C until weight is constant. Weigh oven-dry clod in air (WODC) and in water (WODCW) and record weights.

9. If clod contains >5% rock fragments by weight, remove them from clod. Place clod in a beaker and place on hot plate. Cover hot plate with a liquid vapor trap. Use a fume hood. Heat clod on hot plate in excess of 200°C for 3 to 4 h. The plastic coating disintegrates at temperatures above 200°C. After heating, clod should appear black and charred. Remove clod from hot plate, lightly coat with liquid detergent, and add hot water.

10. Wet sieve the cool soil through a 2-mm, square-hole sieve. Dry and record weight (RF) of rock fragments that are retained on the sieve. Determine rock fragment density by weighing them in air to obtain their mass and in water to obtain their volume. If rock fragments are porous and have a density similar to soil sample, do not correct clod mass and volume measurement for rock fragments. Correct for rock fragments if these fragments can withstand breakdown when dry soil is placed abruptly in water or calgon.

11. Correct bulk density for weight and volume of plastic coating. The coating has an air-dry density of ≈ 1.3g cm^{-3}. The coating loses 10 to 20% of its air-dry weight when dried in oven at 110°C.

5.25.7 Calculations

$$Db_{33} = [WODC - RF - ODPC – TAG]/\{[(CC3 - WMCW)/WD] - (RF/PD) - (MPC1/1.3)\}$$

where:

Db_{33} = Bulk density in g cc-1 of <2 mm fabric at 33 kPa tension
WODC = Weight of oven-dry coated clod
RF = Weight of rock fragments
TAG = Weight of tag and wire
ODPC = MPC1 x 0.85, weight of oven-dry plastic coat
CC3 = Weight of equilibrated clod after four additional plastic coats
WD = Water density
PD = Density of rock fragments
MPC1 = Weight of plastic coat before oven-drying
WMCW = Weight in water of coated clod equilibrated at 33-kPa tension

$$MPC1 = \{[(CC2 - CC1) + FCE] \times RV\} + (CC3-WMC)$$

where:

MPC1 = Weight of plastic coat before oven-drying
CC2 = Weight of clod after three laboratory plastic coats
CC1 = Weight of clod before three laboratory plastic coats
WMC = Weight of coated clod equilibrated at 33-kPa tension
RV = Percent estimate of remaining clod volume after cutting to obtain flat surface (≈ 80%)

$$FCE = 1.5 \times [(CC2 - CC1)/3]$$

where:

FCE = Estimate of field-applied plastic coat

$$Db_{od} = [WODC – RF – ODPC - TAG]/ \{[(WODC - WODCW)/WD] - (RF/PD) - (MPC1/1.3)\}$$

where:

Db_{od} = Bulk density in g cc-1 <2 mm fabric, oven-dry fabric
WODCW = Weight of oven-dry clod coated in water

$$W_{33} = \{[(CC3 – MPC1) – (WODC - ODPC)]/[(WODC - RF - ODPC – TAG)]\} \times 100$$

where:

W_{33} = Percent water weight retained at 33-kPa tension

5.25.8 Report

Bulk density is reported to the nearest 0.01g cm^{-3}.

5.25.9 References

Blake, G.R., and K.H. Hartge. 1986. Bulk density. p. 363-382. In A. Klute (ed.) Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

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Appendix A

Sample Laboratory Forms

Example Tracking Form

Date Received	Sample Type	Sample ID Number	Site ID Number	Laboratory Tracking #	Notes

Water Chemistry Bench Sheet

Project Name/Number_____ Analyst_____

BATCH_ID	SITE_ID	VISIT_NO	SAMPLE_ID	Lab ID	SAMPLE_TYPE	DATE_COLLECTED	DATE_RECEIVED	STATE/TRIBE	REGION	DATE ANALYZED	Technician's Initials	FLAG	Conductivity	pH	NH3	NO3-,NO2-	TN	TP	COMMENTS

Water Chemistry Quality Assurance Bench Sheet

Project Name/Number__________________ Analyst__________________

BATCH_ID	SAMPLEID	REPNUMBER	MATRIX	RESULT_TYPE	PARAMETER	MEASURE	UNIT	DILUTION FACTOR	TRUEVALUE	PERCENT	PERCENTTYPE	ACCEPTANCERANGE	FLAG

Diatom Enumeration Data Sheet

Site ID #_______________ Sample ID #_______________

Lab # _________________ Date Collected_____________

Slide Prepared by _______________ Concentration Factor____

Volume___________________ Steps__________________

Slide Identified by _______________

Notes____

Taxa	**Count Data**					
	Transect 1	**Transect 2**	**Transect 3**	**Transect 4**	**Transect 5**	**Transect 6**

Soft Algae Enumeration Data Sheet

Site ID #_______________ Sample ID #_______________ Lab # __________________

Date Collected__________ Prepared by _______________ Identified by _____________

Taxa	Count Data		
	Natural Count	**Sedgewick-Rafter**	**Palmer- Maloney**

Chlorophyll *a* Data Sheet

Lab ID # _____ Analyst Initials _____

Comments																					
CHLA_DUP																					
CHLA																					
Corrected_CHLA_DU																					
Corrected_CHLA																					
Volume correction (m																					
Dilution factor																					
Raw_CHLA_DUP																					
Raw_CHLA																					
Flag																					
Filtered volume (ml)																					
Date analyzed																					
REGION																					
STATE/TRIBE																					
DATE_RECEIVED																					
DATE_COLLECTED																					
SAMPLE_TYPE																					
Lab ID																					
SAMPLE_ID																					
VISIT_NO																					
SITE_ID																					
BATCH_ID																					

Chlorophyll a Quality Assurance Sheet

Lab ID # ______________ Analyst Initials ______________

BATCH_ID	Rep_Numbe	Lab ID	Sample_Type	Date analyze	Filtered volume (ml)	Flag	Extract Concentration	Extract units	Dilution facto	Comments

Appendix B

NLA and NAWQA Algae Taxa Lists

The NAWQA diatom and non-diatom taxa list can be found at the following website:
http://diatom.acnatsci.org/nawqa/taxalist.asp

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Achnanthes arenaria Amossé
Achnanthes coarctata (Brébisson) Grunow
Achnanthes convergens Kobayasi
Achnanthes curtissima Carter
Achnanthes delicatissima Simonsen
Achnanthes depressa (Cleve) Hustedt
Achnanthes gracillima Hustedt
Achnanthes imperfecta Schimanski
Achnanthes impexa Lange-Bertalot
Achnanthes kriegeri Krasske
Achnanthes lemmermannii Hustedt
Achnanthes levanderi Hustedt
Achnanthes minuscula Hustedt
Achnanthes nitidiformis Lange-Bertalot
Achnanthes nodosa Cleve
Achnanthes nodosa Cleve
Achnanthes oblongella Østrup
Achnanthes pseudoswazi Carter
Achnanthes rosenstockii Lange-Bertalot
Achnanthes rossii Hustedt
Achnanthes rostellata Cleve-Euler
Achnanthes spp.
Achnanthes stolida (Krasske) Krasske
Achnanthes subhudsonis var. kraeuselii (Cholnoky) Cholnoky
Achnanthes submarina Hustedt
Achnanthes trinodis (Ralfs) Grunow
Achnanthes ziegleri Lange-Bertalot
Achnanthidium affine (Grunow) Czarnecki
Achnanthidium altergracillima (Lange-Bertalot) Round et Bukhtiyarova
Achnanthidium biasolettianum (Kützing) Bukhtiyarova
Achnanthidium catenatum (Bily et Marvan) Lange-Bertalot

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Achnanthidium deflexum (Reimer) Kingston
Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot
Achnanthidium exiguum (Grunow) Czarnecki
Achnanthidium exiguum var. 1 NLS PJG
Achnanthidium exiguum var. constrictum (Grunow) Andresen, Stoermer et Kreis
Achnanthidium exiguum var. elliptica Hustedt
Achnanthidium exiguum var. heterovalvum (Krasske) Czarnecki
Achnanthidium exilis (Kützing) Round et Bukhtiyarova
Achnanthidium lapidosum (Krasske) Kobayasi
Achnanthidium macrocephalum (Hustedt) Round et Bukhtiyarova
Achnanthidium minutissimum (Kützing) Czarnecki
Achnanthidium rivulare Potapova et Ponader
Achnanthidium saprophila (Kobayasi et Mayama) Round et Bukhtiyarova
Achnanthidium spp.
Achnanthidium strictum Reichardt
Actinella punctata Lewis
Actinocyclus normanii (Gregory) Hustedt
Adlafia bryophila (Petersen) Lange-Bertalot
Adlafia minuscula (Grunow) Lange-Bertalot
Adlafia minuscula var. muralis (Grunow) Lange-Bertalot
Adlafia multnomahii Morales et Le
Adlafia spp.
Adlafia suchlandtii (Hustedt) Lange-Bertalot
Amphicampa eruca Ehrenberg
Amphipleura kriegeriana (Krasske) Hustedt
Amphipleura pellucida (Kützing) Kützing
Amphipleura spp.
Amphora acutiuscula Kützing
Amphora coffeaeformis (Agardh) Kützing
Amphora commutata Grunow
Amphora copulata (Kützing) Schoeman et Archibald
Amphora delicatissima Krasske
Amphora exigua Gregory
Amphora fogediana Krammer
Amphora holsatica Hustedt
Amphora montana Krasske
Amphora ovalis (Kützing) Kützing

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Amphora pediculus (Kützing) Grunow
Amphora robusta Gregory
Amphora spp.
Amphora strigosa Hustedt
Amphora subcapitata (Kisselev) Hustedt
Amphora thumensis (Mayer) Krieger
Amphora veneta Kützing
Aneumastus minor (Hustedt) Lange-Bertalot
Aneumastus tusculus (Ehrenberg) Mann et Stickle
Anomoeoneis costata (Kützing) Hustedt
Anomoeoneis follis (Ehrenberg) Cleve
Anomoeoneis sphaerophora (Kützing) Pfitzer
Anomoeoneis sphaerophora fo. costata (Kützing) Schmidt
Anomoeoneis sphaerophora fo. sculpta (Ehrenberg) Krammer
Anomoeoneis spp.
Anomoeoneis styriaca (Grunow) Hustedt
Anorthoneis excentrica (Donkin) Grunow
Astartiella bahusiensis (Grunow) Witkowski, Lange-Bertalot et Metzeltin
Asterionella formosa Hassal
Asterionella ralfsii Smith
Asterionella spp.
Aulacoseira alpigena (Grunow) Krammer
Aulacoseira ambigua (Grunow) Simonsen
Aulacoseira canadensis (Hustedt) Simonsen
Aulacoseira crassipunctata Krammer
Aulacoseira crenulata (Ehrenberg) Thwaites
Aulacoseira distans var. nivalis (Smith) Haworth
Aulacoseira distans var. nivaloides (Camburn) Haworth
Aulacoseira granulata (Ehrenberg) Simonsen
Aulacoseira granulata fo. valida (Hustedt) Simonsen
Aulacoseira granulata var. angustissima (Müller) Simonsen
Aulacoseira herzogii (Lemmermann) Simonsen
Aulacoseira islandica (Müller) Simonsen
Aulacoseira italica var. tenuissima (Grunow) Simonsen
Aulacoseira italica var. valida (Grunow) Simonsen
Aulacoseira lacustris (Grunow) Krammer
Aulacoseira laevissima (Grunow) Krammer

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Aulacoseira lirata (Ehrenberg) Ross
Aulacoseira nygaardii Camburn
Aulacoseira perglabra (Østrup) Haworth
Aulacoseira perglabra var. florinae (Camburn) Haworth
Aulacoseira pfaffiana (Reinsch) Krammer
Aulacoseira spp.
Aulacoseira subarctica (Müller) Haworth
Aulacoseira tenella (Nygaard) Simonsen
Aulacoseira tenuior (Grunow) Krammer
Aulacoseira tethera Haworth
Aulacoseira valida (Grunow) Krammer
Bacillaria paradoxa Gmelin
Bacillaria spp.
Biremis circumtexta (Meister ex Hustedt) Lange-Bertalot et Witkowski
Brachysira apiculata (Boyer) Lange-Bertalot et Moser
Brachysira aponina Kützing
Brachysira brebissonii Ross
Brachysira elliptica Metzeltin et Lange-Bertalot
Brachysira microcephala (Grunow) Compère
Brachysira serians (Brébisson) Round et Mann
Brachysira spp.
Caloneis alpestris (Grunow) Cleve
Caloneis amphisbaena (Bory) Cleve
Caloneis bacillum (Grunow) Cleve
Caloneis borealis Carter
Caloneis branderii (Hustedt) Krammer
Caloneis clevei (Lagerstedt) Cleve
Caloneis hyalina Hustedt
Caloneis lewisii Patrick
Caloneis macedonica (Gregory) Krammer
Caloneis molaris (Grunow) Krammer
Caloneis schumanniana (Grunow) Cleve
Caloneis schumanniana var. biconstricta (Grunow) Reichelt
Caloneis silicula (Ehrenberg) Cleve
Caloneis spp.
Caloneis tenuis (Gregory) Krammer
Caloneis undulata (Gregory) Krammer

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Caloneis ventricosa var. subundulata (Grunow) Patrick
Caloneis westii (Smith) Hendey
Campylodiscus bicostatus Smith
Campylodiscus clypeus Ehrenberg
Campylodiscus hibernicus Ehrenberg
Campylodiscus levanderi Hustedt
Campylodiscus noricus Ehrenberg
Campylodiscus spp.
Campylopyxis germainii Mann
Campylostylus normannianus (Greville) Gerloff, Natour et Rivera
Capartogramma crucicula (Grunow ex Cleve) Ross
Cavinula cocconeiformis (Gregory ex Greville) Mann et Stickle
Cavinula jaernefelti (Hustedt) Mann et Stickle
Cavinula lacustris (Gregory) Mann et Stickle
Cavinula lapidosa (Krasske) Lange-Bertalot
Cavinula pseudoscutiformis (Grunow ex Schmidt) Mann et Stickle
Cavinula pusio (Cleve) Lange-Bertalot
Cavinula scutelloides (Smith) Lange-Bertalot et Metzeltin
Cavinula scutiformis (Grunow ex Schmidt) Mann et Stickle
Cavinula spp.
Cavinula variostriata (Krasske) Lange-Bertalot
Cavinula weinzierlii (Schimanski) Czarnecki
Chaetoceros spp.
Chamaepinnularia begeri (Krasske) Lange-Bertalot
Chamaepinnularia bremensis (Hustedt) Lange-Bertalot
Chamaepinnularia evanida (Hustedt) Lange-Bertalot
Chamaepinnularia krookii (Grunow) Lange-Bertalot et Krammer
Chamaepinnularia mediocris (Krasske) Lange-Bertalot
Chamaepinnularia schauppiana Lange-Bertalot et Metzeltin
Chamaepinnularia soehrensis (Krasske) Lange-Bertalot et Krammer
Chamaepinnularia soehrensis var. hassiaca (Krasske) Lange-Bertalot
Chamaepinnularia soehrensis var. muscicola (Petersen) Lange-Bertalot et Krammer
Chamaepinnularia sp. 1 NLS GDL
Chamaepinnularia spp.
Cocconeis diminuta Pantocsek
Cocconeis disculus (Schumann) Cleve
Cocconeis neodiminuta Krammer

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Cocconeis neothumensis Krammer
Cocconeis pediculus Ehrenberg
Cocconeis peltoides Hustedt
Cocconeis placentula Ehrenberg
Cocconeis placentula var. klinoraphis Geitler
Cocconeis placentula var. rouxii (Héribaud et Brun in Héribaud) Cleve
Cocconeis pseudolineata (Geitler) Lange-Bertalot
Cocconeis pseudothumensis Reichardt
Cocconeis scutellum Ehrenberg
Cocconeis spp.
Coscinodiscus spp.
Cosmioneis spp.
Craticula accomoda (Hustedt) Mann
Craticula acidoclinata Lange-Bertalot et Metzeltin
Craticula ambigua (Ehrenberg) Mann
Craticula buderi (Hustedt) Lange-Bertalot
Craticula citrus (Krasske) Reichardt
Craticula cuspidata (Kützing) Mann
Craticula halophila (Grunow) Mann
Craticula halophilioides (Hustedt) Lange-Bertalot
Craticula molestiformis (Hustedt) Lange-Bertalot
Craticula riparia (Hustedt) Lange-Bertalot
Craticula riparia var. mollenhaueri Lange-Bertalot
Craticula sardiniensis (Lange-Bertalot, Cavacini, Tagliaventi et Alfinito) Morales et Le
Craticula spp.
Craticula submolesta (Hustedt) Lange-Bertalot
Craticula vixnegligenda Lange-Bertalot
Ctenophora pulchella (Ralfs ex Kützing) Williams et Round
Ctenophora pulchella var. lacerata (Hustedt) Bukhtiyarova
Cyclostephanos costatilimbus Stoermer, Håkansson et Theriot
Cyclostephanos damasii (Hustedt) Stoermer et Håkansson
Cyclostephanos dubius (Fricke) Round
Cyclostephanos invisitatus (Hohn et Hellerman) Theriot, Stoermer et Håkansson
Cyclostephanos spp.
Cyclostephanos tholiformis Stoermer, Håkansson et Theriot
Cyclotella antiqua Smith
Cyclotella atomus Hustedt

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Cyclotella bodanica var. affinis (Grunow) Cleve-Euler
Cyclotella bodanica var. lemanica O. Müller (Bachmann)
Cyclotella comensis Grunow et Van Heurck
Cyclotella cyclopuncta Håkansson
Cyclotella delicatula Hustedt
Cyclotella distinguenda Hustedt
Cyclotella distinguenda var. unipunctata (Hustedt) Håkansson et Carter
Cyclotella gamma Skvortzow
Cyclotella glabriuscula (Grunow) Håkansson
Cyclotella hakanssoniae Wendker
Cyclotella krammeri Håkansson
Cyclotella kuetzingiana Thwaites
Cyclotella meneghiniana Kützing
Cyclotella michiganiana Skvortzow
Cyclotella michiganiana var. 1 NLS GDL
Cyclotella ocellata Pantocsek
Cyclotella planctonica Brunnthaler
Cyclotella polymorpha Meyer et Håkansson
Cyclotella quadrijuncta (Schröter) von Keissler
Cyclotella schumannii (Grunow) Håkansson
Cyclotella spp.
Cyclotella striata (Kützing) Grunow
Cyclotella tripartita Håkansson
Cylindrotheca gracilis (Brébisson) Grunow
Cymatopleura elliptica (Brébisson) Smith
Cymatopleura solea (Brébisson) Smith
Cymatopleura spp.
Cymbella acuta (Schmidt in Schmidt et al.) Cleve
Cymbella aequalis Smith
Cymbella affinis Kützing
Cymbella alpina Grunow
Cymbella amphicephala Nägeli ex Kützing
Cymbella amphioxys (Kützing) Cleve
Cymbella angustata (Smith) Cleve
Cymbella aspera (Ehrenberg) Peragallo
Cymbella austriaca Grunow
Cymbella budayana Pantocsek

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Cymbella caespitosa Brun
Cymbella cistula (Ehrenberg) Kirchner
Cymbella cuspidata Kützing
Cymbella cymbiformis Agardh
Cymbella delicatula Kützing
Cymbella diluviana (Krasske) Florin
Cymbella ehrenbergii Kützing
Cymbella elginensis Krammer
Cymbella gracilis (Ehrenberg) Kützing
Cymbella hauckii Van Heurck
Cymbella helvetica Kützing
Cymbella heteropleura (Ehrenberg) Kützing
Cymbella hustedtii Krasske
Cymbella hybrida Grunow ex Cleve
Cymbella incerta (Grunow) Cleve
Cymbella incerta var. crassipunctata Krammer
Cymbella laevis Nägeli ex Kützing
Cymbella lanceolata (Agardh) Agardh
Cymbella lapponica Grunow
Cymbella lata Grunow
Cymbella leptoceros (Ehrenberg) Kützing
Cymbella mesiana Cholnoky
Cymbella mexicana (Ehrenberg) Cleve
Cymbella mexicana var. janischii (Schmidt) Reimer
Cymbella moelleriana Grunow
Cymbella naviculiformis Auerswald ex Héribaud
Cymbella obscura Krasske
Cymbella paucistriata Cleve-Euler
Cymbella proxima Reimer
Cymbella pusilla Grunow
Cymbella reinhardtii Grunow
Cymbella rupicola Grunow
Cymbella similis Krasske
Cymbella simonsenii Krammer
Cymbella smithii Rabenhorst
Cymbella spp.
Cymbella stauroneiformis Lagerstedt

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Cymbella subaequalis fo. krasskei (Foged) Reimer
Cymbella subaequalis Grunow
Cymbella subcuspidata Krammer
Cymbella subturgidula Krammer
Cymbella tumidula Grunow ex Schmidt
Cymbella tumidula var. lancettula Krammer
Cymbella turgida Gregory
Cymbella turgidula Grunow
Cymbella tynnii Krammer
Cymbellonitzschia diluviana Hustedt
Cymbopleura cuspidata Kützing
Decussata placenta (Ehrenberg) Lange-Bertalot et Metzeltin
Denticula elegans Kützing
Denticula kuetzingii Grunow
Denticula kuetzingii Grunow
Denticula spp.
Denticula subtilis Grunow
Denticula tenuis Kützing
Denticula valida (Pedicino) Grunow
Diadesmis confervacea Kützing
Diadesmis contenta (Grunow ex Van Heurck) Mann
Diadesmis contenta var. biceps (Grunow) Hamilton
Diadesmis gallica Smith
Diadesmis irata (Krasske) Moser, Lange-Bertalot et Metzeltin
Diadesmis laevissima (Cleve) Mann
Diadesmis perpusilla (Grunow) Mann
Diadesmis spp.
Diatoma anceps (Ehrenberg) Kirchner
Diatoma hyemalis (Roth) Heiberg
Diatoma mesodon (Ehrenberg) Kützing
Diatoma moniliformis Kützing
Diatoma spp.
Diatoma tenuis Agardh
Diatoma tenuis var. elongatum Lyngbye
Diatoma vulgaris Bory
Diatoma vulgaris var. breve Grunow
Diploneis boldtiana Cleve

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Diploneis elliptica (Kützing) Cleve
Diploneis finnica (Ehrenberg) Cleve
Diploneis litoralis (Donkin) Cleve
Diploneis marginestriata Hustedt
Diploneis modica Hustedt
Diploneis oblongella (Nägeli ex Kützing) Ross
Diploneis oculata (Brébisson) Cleve
Diploneis ovalis (Hilse ex Rabenhorst) Cleve
Diploneis parma Cleve
Diploneis parma Cleve
Diploneis peterseni Hustedt
Diploneis pseudovalis Hustedt
Diploneis puella (Schumann) Cleve
Diploneis smithii (Brébisson ex Smith) Cleve
Diploneis smithii var. pumila (Grunow) Hustedt
Diploneis spp.
Diploneis subovalis Cleve
Discostella asterocostata (Xie, Lin et Cai) Houk et Klee
Discostella glomerata (Bachmann) Houk et Klee
Discostella spp.
Discostella stelligera (Hustedt) Houk et Klee
Ellerbeckia arenaria (Moore) Crawford
Encyonema auerswaldii Rabenhorst
Encyonema gaeumanii (Meister) Krammer
Encyonema gracile Rabenhorst
Encyonema hebridicum Grunow ex Cleve
Encyonema latens (Krasske) Mann
Encyonema lunatum (Smith) Van Heurck
Encyonema minutum (Hilse) Mann
Encyonema muelleri (Hustedt) Mann
Encyonema norvegicum (Grunow) Mills
Encyonema norvegicum (Grunow) Mills
Encyonema obscurum (Krasske) Mann
Encyonema obscurum var. alpina Krammer
Encyonema paucistriatum (Cleve-Euler) Mann
Encyonema perpusillum (Cleve) Mann
Encyonema prostratum (Berkeley) Kützing

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Encyonema reichardtii (Krammer) Mann
Encyonema spp.
Encyonema triangulum (Ehrenberg) Kützing
Encyonema tumida (Brébisson ex Kützing) Mann
Encyonema ventricosum Agardh
Encyonopsis cesatii (Rabenhorst) Krammer
Encyonopsis delicatissima (Hustedt) Krammer
Encyonopsis descripta (Hustedt) Krammer
Encyonopsis evergladianum Krammer
Encyonopsis falaisensis (Grunow) Krammer
Encyonopsis krammeri Reichardt
Encyonopsis microcephala (Grunow) Krammer
Encyonopsis minuta Krammer et Reichardt
Encyonopsis spp.
Encyonopsis subminuta Krammer et Reichardt
Entomoneis alata (Ehrenberg) Ehrenberg
Entomoneis costata (Hustedt) Reimer
Entomoneis ornata (Bailey) Reimer
Entomoneis spp.
Epithemia adnata (Kützing) Brébisson
Epithemia adnata var. porcellus (Kützing) Patrick
Epithemia argus (Ehrenberg) Kützing
Epithemia reichelti Fricke
Epithemia smithii Carruthers
Epithemia sorex Kützing
Epithemia spp.
Epithemia turgida (Ehrenberg) Kützing
Epithemia turgida var. granulata (Ehrenberg) Hustedt
Epithemia turgida var. westermannii (Ehrenberg) Grunow
Eucocconeis alpestris (Brun) Lange-Bertalot
Eucocconeis flexella (Kützing) Cleve
Eucocconeis laevis (Østrup) Lange-Bertalot
Eucocconeis laevis var. diluviana (Hustedt) Lange-Bertalot
Eucocconeis spp.
Eunotia arculus (Grunow) Lange-Bertalot et Nörpel
Eunotia arcus Ehrenberg
Eunotia bactriana Ehrenberg

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Eunotia bidentula Smith
Eunotia bigibba Kützing
Eunotia bilunaris (Ehrenberg) Mills
Eunotia camelus Ehrenberg
Eunotia carolina Patrick
Eunotia cf. paludosa NLA EWT Grunow
Eunotia cf. paludosa NLA JPK Grunow
Eunotia cf. pirla NLS DME Carter et Flower
Eunotia cf. sudetica NLA DB Müller
Eunotia circumborealis Lange-Bertalot et Nörpel
Eunotia croatana Siver, Hamilton et Morales
Eunotia curvata fo. bergii Woodhead et Tweed
Eunotia diodon Ehrenberg
Eunotia elegans Østrup
Eunotia exigua (Brébisson ex Kützing) Rabenhorst
Eunotia faba (Ehrenberg) Grunow
Eunotia fallax Cleve
Eunotia fallax var. gracillima Krasske
Eunotia fallax var. groenlandica (Grunow) Lange-Bertalot et Nörpel
Eunotia flexuosa Brébisson ex Kützing
Eunotia formica Ehrenberg
Eunotia glacialis Meister
Eunotia hemicyclus (Ehrenberg) Karsten
Eunotia hexaglyphis Ehrenberg
Eunotia implicata Nörpel, Lange-Bertalot et Alles
Eunotia incisa Smith ex Gregory
Eunotia inflata (Grunow) Nörpel-Schempp et Lange-Bertalot
Eunotia intermedia (Krasske ex Hustedt) Nörpel et Lange-Bertalot
Eunotia lapponica Grunow ex Cleve
Eunotia luna Ehrenberg
Eunotia meisteri Hustedt
Eunotia microcephala Krasske ex Hustedt
Eunotia minor (Kützing) Grunow
Eunotia muscicola var. tridentula Nörpel et Lange-Bertalot
Eunotia naegelii Migula
Eunotia neofallax Nörpel-Schempp et Lange-Bertalot
Eunotia nymanii Grunow

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Eunotia nymanniana Grunow (sensu Hustedt)
Eunotia paludosa Grunow
Eunotia paludosa var. trinacria (Krasske) Nörpel
Eunotia parallela Ehrenberg
Eunotia pectinalis (Müller) Rabenhorst
Eunotia perpusilla Grunow
Eunotia pirla Carter et Flower
Eunotia praerupta Ehrenberg
Eunotia praerupta var. bigibba (Kützing) Grunow
Eunotia praerupta var. curta Grunow
Eunotia pseudoparalleloides (Grunow) Nörpel-Schempp et Lange-Bertalot
Eunotia rabenhorstii Grunow
Eunotia rhomboidea Hustedt
Eunotia rhynchocephala Hustedt
Eunotia rhynchocephala var. satelles Nörpel et Lange-Bertalot
Eunotia seminulum Nörpel-Schempp et Lange-Bertalot
Eunotia septentrionalis Østrup
Eunotia serra Ehrenberg
Eunotia serra var. diadema (Ehrenberg) Patrick
Eunotia serra var. tetraodon Nörpel
Eunotia silvahercynia Nörpel, Van Sull et Lange-Bertalot
Eunotia siolii Hustedt
Eunotia soleirolii (Kützing) Rabenhorst
Eunotia spp.
Eunotia subarcuatoides Alles, Nörpel et Lange-Bertalot
Eunotia sudetica Müller
Eunotia tenella (Grunow) Hustedt
Eunotia triodon Ehrenberg
Eunotia valida Hustedt
Eunotia yanomami Metzeltin et Lange-Bertalot
Eunotia zasuminensis (Cabejszekowna) Körner
Eunotia zygodon Ehrenberg
Fallacia auriculata (Hustedt) Mann
Fallacia cryptolyra (Brockmann) Stickle et Mann
Fallacia enigmatica (Germain) Lange-Bertalot
Fallacia indifferens (Hustedt) Mann
Fallacia insociabilis (Krasske) Stickle et Mann

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Fallacia lenzii (Hustedt) Lange-Bertalot
Fallacia litoricola (Hustedt) Mann
Fallacia lucinensis (Hustedt) Mann in Round, Crawford et Mann
Fallacia monoculata (Hustedt) Mann
Fallacia omissa (Hustedt) Mann
Fallacia pygmaea (Kützing) Stickle et Mann
Fallacia spp.
Fallacia subforcipata (Hustedt) Mann
Fallacia subhamulata (Grunow) Mann
Fallacia tenera (Hustedt) Mann
Fistulifera pelliculosa (Brébisson ex Kützing) Lange-Bertalot
Fistulifera saprophila (Lange-Bertalot et Bonik) Lange-Bertalot
Fragilaria acidobiontica Charles
Fragilaria acutirostrata Metzeltin et Lange-Bertalot
Fragilaria alpestris Krasske
Fragilaria amphicephala (Kützing) Lange-Bertalot
Fragilaria capucina Desmazières
Fragilaria capucina var. mesolepta Rabenhorst
Fragilaria capucina var. perminuta (Grunow) Lange-Bertalot
Fragilaria construens var. exigua (Smith) Schulz
Fragilaria crotonensis Kitton
Fragilaria cyclopum (Brutschy) Lange-Bertalot
Fragilaria distans (Grunow) Bukhtiyarova
Fragilaria exigua Grunow
Fragilaria famelica (Kützing) Lange-Bertalot
Fragilaria germainii Reichardt et Lange-Bertalot
Fragilaria heidenii Østrup
Fragilaria neoproducta Lange-Bertalot
Fragilaria nitzschioides Grunow
Fragilaria pinnata var. acuminata Mayer
Fragilaria radians (Kützing) Lange-Bertalot
Fragilaria rhabdosoma Ehrenberg
Fragilaria sepes Ehrenberg
Fragilaria spp.
Fragilaria subconstricta Østrup
Fragilaria tenuistriata Østrup
Fragilaria vaucheriae (Kützing) Petersen

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Fragilaria zeilleri var. elliptica Gasse
Fragilariforma bicapitata (Mayer) Williams et Round
Fragilariforma constricta (Ehrenberg) Williams et Round
Fragilariforma constricta fo. stricta (Cleve) Poulin
Fragilariforma constricta var. trinodis (Hustedt) Hamilton
Fragilariforma hungarica var. tumida (Cleve-Euler) Hamilton
Fragilariforma lata (Cleve-Euler) Williams et Round
Fragilariforma polygonata (Cleve-Euler) Kingston, Sherwood et Bengston
Fragilariforma spinulosa (Patrick) Metzeltin et Lange-Bertalot
Fragilariforma spp.
Fragilariforma virescens (Ralfs) Williams et Round
Frustulia amphipleuroides (Grunow) Cleve-Euler
Frustulia bahlsii Edlund et Brant
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer
Frustulia erifuga Lange-Bertalot et Krammer
Frustulia krammeri Lange-Bertalot et Metzeltin
Frustulia pseudomagaliesmontana Camburn et Charles
Frustulia quadrisinuata Lange-Bertalot in Lange-Bertalot et Metzeltin
Frustulia saxonica Rabenhorst
Frustulia spp.
Frustulia viridula Kützing
Frustulia vulgaris (Thwaites) De Toni
Frustulia weinholdii Hustedt
Geissleria acceptata (Hustedt) Lange-Bertalot et Metzeltin
Geissleria aikenensis (Patrick) Torgan et Olivera
Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin
Geissleria dolomitica (Bock) Lange-Bertalot et Metzeltin
Geissleria ignota (Krasske) Lange-Bertalot et Metzeltin
Geissleria paludosa (Hustedt) Lange-Bertalot et Metzeltin
Geissleria schoenfeldii (Hustedt) Lange-Bertalot et Metzeltin
Geissleria spp.
Gomphoneis eriense var. variabilis Kociolek et Stoermer
Gomphoneis geitleri Kociolek et Stoermer
Gomphoneis herculeana (Ehrenberg) Cleve
Gomphoneis herculeana var. robusta (Grunow) Cleve
Gomphoneis minuta Kociolek et Stoermer
Gomphoneis spp.

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Gomphoneis transsilvanica (Pantocsek) Krammer in Krammer et Lange-Bertalot
Gomphonema acuminatum Ehrenberg
Gomphonema acuminatum var. coronatum (Ehrenberg) Rabenhorst
Gomphonema acuminatum var. pusillum Grunow
Gomphonema acutiusculum (Müller) Cleve-Euler
Gomphonema affine Kützing
Gomphonema amoenum Lange-Bertalot
Gomphonema angustatum (Kützing) Rabenhorst
Gomphonema angustum Agardh
Gomphonema anjae Lange-Bertalot et Reichardt
Gomphonema apicatum Ehrenberg
Gomphonema apuncto Wallace
Gomphonema augur Ehrenberg
Gomphonema augur var. turris (Ehrenberg) Lange-Bertalot
Gomphonema auritum Braun
Gomphonema bohemicum Reichardt et Fricke
Gomphonema camburnii Lange-Bertalot
Gomphonema capitatum Ehrenberg
Gomphonema carolinense Hagelstein
Gomphonema clavatum Ehrenberg
Gomphonema clevei Fricke in Schmidt et al.
Gomphonema contraturris Lange-Bertalot et Reichardt
Gomphonema dichotomum Kützing
Gomphonema drutelingense Reichardt
Gomphonema exilissimum (Grunow) Lange-Bertalot
Gomphonema germainii Kociolek et Stoermer
Gomphonema gibba Wallace
Gomphonema gracile Ehrenberg emend Van Heurck
Gomphonema gracile fo. cymbelloides (Grunow ms. in Cleve) Mayer
Gomphonema hebridense Gregory
Gomphonema innocens Reichardt
Gomphonema insigne Gregory
Gomphonema intricatum Kützing
Gomphonema intricatum var. vibrio (Ehrenberg) Cleve
Gomphonema kobayashiae Metzeltin et Lange-Bertalot
Gomphonema kobayasii Kociolek et Kingston
Gomphonema lagenula Kützing

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Gomphonema longilineare Reichardt
Gomphonema maclaughlinii Reichardt
Gomphonema mexicanum Grunow ex Van Heurck
Gomphonema micropumilum Reichardt
Gomphonema micropus Kützing
Gomphonema minusculum Krasske
Gomphonema minutum (Agardh) Agardh
Gomphonema olivaceoides Hustedt
Gomphonema olivaceum (Lyngbye) Kützing
Gomphonema parvulius (Lange-Bertalot et Reichardt) Lange-Bertalot et Reichardt
Gomphonema parvulum (Kützing) Kützing
Gomphonema patrickii Kociolek et Stoermer
Gomphonema patrickii Kociolek et Stoermer
Gomphonema productum (Grunow) Lange-Bertalot et Reichardt
Gomphonema pseudoaugur Lange-Bertalot
Gomphonema pseudotenellum Lange-Bertalot
Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot
Gomphonema pygmaeum Kociolek et Stoermer
Gomphonema quadripunctatum (Østrup) Wislouch
Gomphonema rhombicum Fricke
Gomphonema sarcophagus Gregory
Gomphonema sphaerophorum Ehrenberg
Gomphonema spp.
Gomphonema subclavatum (Grunow) Grunow
Gomphonema subclavatum var. commutatum (Grunow) Mayer
Gomphonema subclavatum var. mexicanum (Grunow) Patrick
Gomphonema subtile Ehrenberg
Gomphonema subtile var. sagitta (Schumann) Grunow ex Van Heurck
Gomphonema truncatum Ehrenberg
Gomphonema truncatum var. capitatum (Ehrenberg) Patrick
Gomphonema turgidum Ehrenberg
Gomphonema turris Ehrenberg
Gomphonema utae Lange-Bertalot et Reichardt
Gomphonema variostriatum Camburn et Charles
Gomphonema vibrioides Reicherdt et Lange-Bertalot
Gomphonitzschia spp.
Gomphosphenia grovei (Schmid) Lange-Bertalot

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Gomphosphenia grovei var. lingulata (Hustedt) Lange-Bertalot
Gomphosphenia lingulatiformis (Lange-Bertalot et Reichardt) Lange-Bertalot
Gomphosphenia spp.
Gomphosphenia tackei (Hustedt) Lange-Bertalot
Gyrosigma acuminatum (Kützing) Rabenhorst
Gyrosigma attenuatum (Kützing) Rabenhorst
Gyrosigma balticum (Ehrenberg) Rabenhorst
Gyrosigma macrum (Smith) Griffith et Henfrey
Gyrosigma nodiferum (Grunow) Reimer
Gyrosigma obscurum (Smith) Griffith et Henfrey
Gyrosigma obtusatum (Sullivant et Wormley) Boyer
Gyrosigma parkerii (Harrison) Elmore
Gyrosigma scalproides (Rabenhorst) Cleve
Gyrosigma sciotoense (Sullivan et Wormley) Cleve
Gyrosigma spp.
Gyrosigma strigilis (Smith) Cleve
Hannaea arcus (Ehrenberg) Patrick
Hantzschia amphioxys (Ehrenberg) Grunow
Hantzschia distinctepunctata Hustedt
Hantzschia elongata (Hantzsch) Grunow
Hantzschia spectabilis (Ehrenberg) Hustedt
Hantzschia spp.
Hantzschia virgata (Roper) Grunow
Hantzschia vivax (Smith) Peragallo
Haslea spicula (Hickle) Lange-Bertalot
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski
Hippodonta costulata (Grunow) Lange-Bertalot, Metzeltin et Witkowski
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin et Witkowski
Hippodonta lueneburgensis (Grunow) Lange-Bertalot, Metzeltin et Witkowski
Hippodonta spp.
Hippodonta subcostulata (Hustedt) Lange-Bertalot, Metzeltin et Witkowski
Karayevia amoena (Hustedt) Bukhtiyarova
Karayevia bottnica (Cleve) Lange-Bertalot
Karayevia carissima (Lange-Bertalot) Bukhtiyarova
Karayevia clevei (Grunow) Bukhtiyarova
Karayevia clevei var. bottnica (Cleve) Bukhtiyarova
Karayevia clevei var. rostrata (Hustedt) Bukhtiyarova

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Karayevia laterostrata (Hantzsch) Bukhtiyarova
Karayevia ploenensis (Hustedt) Bukhtiyarova
Karayevia spp.
Karayevia suchlandtii (Hustedt) Bukhtiyarova
Kobayasia parasubtilissima (Kobayasi et Nagumo) Lange-Bertalot
Kobayasiella jaegii (Meister) Lange-Bertalot
Kobayasiella spp.
Kobayasiella subtilissima (Cleve) Lange-Bertalot
Kobayasiella venezuelensis Metzeltin et Lange-Bertalot
Krasskella kriegerana (Krasske) Ross et Sims
Lemnicola hungarica (Grunow) Round et Basson
Luticola cohnii (Hilse) Mann
Luticola dismutica (Hustedt) Mann in Round, Crawford et Mann
Luticola goeppertiana (Bleisch) Mann
Luticola mutica (Kützing) Mann
Luticola muticoides (Hustedt) Mann
Luticola nivalis (Ehrenberg) Mann
Luticola nivaloides Johansen
Luticola spp.
Luticola ventricosa (Kützing) Mann
Mastogloia elliptica (Agardh) Cleve
Mastogloia elliptica var. danseii (Thwaites) Cleve
Mastogloia grevillei Smith
Mastogloia lanceolata Thwaites in Smith
Mastogloia pumila (Cleve et Möller; Grunow in Van Heurck) Cleve
Mastogloia pusilla Grunow
Mastogloia smithii Thwaites
Mastogloia smithii var. lacustris Grunow
Mastogloia spp.
Mayamaea atomus (Kützing) Lange-Bertalot
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot
Mayamaea excelsa (Krasske) Lange-Bertalot
Mayamaea recondita (Hustedt) Lange-Bertalot
Mayamaea spp.
Melosira arentii (Kolbe) Nagumo et Kobayasi
Melosira distans var. humilis Cleve-Euler
Melosira undulata (Ehrenberg) Kützing

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Melosira varians Agardh
Meridion circulare (Greville) Agardh
Meridion circulare var. constrictum (Ralfs) Van Heurck
Meridion spp.
Microcostatus krasskei (Hustedt) Johansen et Sray
Microcostatus kuelbsii (Lange-Bertalot) Lange-Bertalot
Microcostatus maceria (Schimanski) Lange-Bertalot
Microcostatus naumannii (Hustedt) Lange-Bertalot
Muelleria gibbula (Cleve) Spaulding et Stoermer
Navicula aboensis (Cleve) Hustedt
Navicula absoluta Hustedt
Navicula amphibola Cleve
Navicula amphiceropsis Lange-Bertalot et Rumrich
Navicula angusta Grunow
Navicula angustata Smith
Navicula antonii Lange-Bertalot
Navicula arctotenelloides Lange-Bertalot et Metzeltin
Navicula arenaria Donkin
Navicula arvensis Hustedt
Navicula arvensis var. major Lange-Bertalot
Navicula aurora Sovereign
Navicula bacilloides Hustedt
Navicula bourrellyivera Lange-Bertalot, Witkowski et Stachura
Navicula brasiliana Cleve
Navicula canalis Patrick
Navicula capitatoradiata Germain
Navicula capitellata Skvortzow
Navicula cari Ehrenberg
Navicula catalanogermanica Lange-Bertalot et Hofmann
Navicula caterva Hohn et Hellerman
Navicula cincta (Ehrenberg) Ralfs
Navicula cocconeiformis var. capitata Krasske
Navicula comoides (Dillwyn; Dillwyn) Peragallo et Peragallo
Navicula concentrica Carter
Navicula constans Hustedt
Navicula constans var. symmetrica Hustedt
Navicula cryptocephala Kützing

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Navicula cryptocephaloides Hustedt
Navicula cryptofallax Lange-Bertalot ex Hofmann
Navicula cryptotenella Lange-Bertalot in Krammer et Lange-Bertalot
Navicula denselineolata (Lange-Bertalot) Lange-Bertalot
Navicula detenta Hustedt
Navicula difficillima Hustedt
Navicula difficillimoides Hustedt
Navicula digitoradiata (Gregory) Ralfs ex Pritchard
Navicula digitoradiata var. rostrata Hustedt
Navicula digitulus Hustedt
Navicula disjuncta Hustedt
Navicula effrenata Krasske
Navicula eidrigiana Carter
Navicula erifuga Lange-Bertalot
Navicula exigua var. signata Hustedt
Navicula exilis Kützing
Navicula facilis Krasske
Navicula farta Hustedt
Navicula festiva Krasske
Navicula fluens Hustedt
Navicula fossalis Krasske
Navicula fossalis var. obsidialis (Hustedt) Lange-Bertalot in Krammer et Lange-Bertalot
Navicula fracta Hustedt
Navicula gallica var. perpusilla (Grunow) Lange-Bertalot
Navicula gastrum var. signata Hustedt
Navicula gerloffi Schimanski
Navicula germainii Wallace
Navicula globulifera Hustedt
Navicula glomus Carter et Bailey-Watts
Navicula goersii Bahls
Navicula gottlandica Grunow
Navicula gregaria Donkin
Navicula hambergii Hustedt
Navicula hasta Pantocsek
Navicula heimansioides Lange-Bertalot
Navicula helensis Schulz
Navicula heufleriana (Grunow) Cleve

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Navicula hintzii Lange-Bertalot
Navicula hintzii Lange-Bertalot
Navicula hoeflerii Cholnoky
Navicula hustedtiana Simonsen
Navicula hustedtii var. obtusa Hustedt
Navicula ignota var. palustris (Hustedt) Lund
Navicula imbricata Bock
Navicula impexa Hustedt
Navicula incertata Hustedt
Navicula ingenua Hustedt
Navicula ingrata Krasske
Navicula integra (Smith) Ralfs
Navicula irmengardis Lange-Bertalot in Lange-Bertalot et Metzeltin
Navicula jaagii Meister
Navicula joubaudii Germain
Navicula kotschyi Grunow
Navicula kriegerii Krasske
Navicula kuelbsii Lange-Bertalot
Navicula lacustris Gregory
Navicula lanceolata (Agardh) Ehrenberg
Navicula lateropunctata Wallace
Navicula laterostrata Hustedt
Navicula laterostrata Hustedt
Navicula laticeps Hustedt
Navicula leistikowii Lange-Bertalot
Navicula leptostriata Jørgensen
Navicula lesmonensis Hustedt
Navicula levanderii Hustedt
Navicula libonensis Schoeman
Navicula lineolata Ehrenberg
Navicula longa (Gregory) Ralfs in Pritchard
Navicula longicephala Hustedt
Navicula ludloviana Schmidt in Schmidt et al.
Navicula lundii Reichardt
Navicula luzonensis Hustedt
Navicula mandumensis Jørgensen
Navicula margalithii Lange-Bertalot

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Navicula medioconvexa Hustedt
Navicula menisculus Schumann
Navicula menisculus var. obtusa Hustedt
Navicula meniscus Schumann
Navicula minima Grunow
Navicula minusculoides Hustedt
Navicula minuta Cleve-Euler
Navicula minutula Smith
Navicula modica Hustedt
Navicula moskalii Metzeltin, Witkowski et Lange-Bertalot
Navicula muraliformis Hustedt
Navicula normaloides Cholnoky
Navicula notha Wallace
Navicula oblonga Østrup
Navicula oblongella Nägeli
Navicula obsidialis Hustedt
Navicula obsoleta Hustedt
Navicula occulta Krasske
Navicula oligotraphenta Lange-Bertalot et Hofmann
Navicula oppugnata Hustedt
Navicula ordinaria Hustedt
Navicula parablis Hohn et Hellerman
Navicula paramutica Bock
Navicula pelliculosa (Brébisson) Hilse
Navicula peregrina (Ehrenberg) Kützing
Navicula peregrinopsis Lange-Bertalot et Witkowski
Navicula perminuta Grunow
Navicula phyllepta Kützing
Navicula phylleptosoma Lange-Bertalot
Navicula porifera (Hustedt) Lange-Bertalot
Navicula porifera var. opportuna Lange-Bertalot
Navicula praeterita Hustedt
Navicula protracta var. elliptica Gallik
Navicula pseudoarvensis Hustedt
Navicula pseudobryophila Hustedt
Navicula pseudolanceolata Lange-Bertalot
Navicula pseudosilicula Hustedt

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Navicula pseudosubtilissima Manguin
Navicula pseudoventralis Hustedt
Navicula pupula var. aquaductae (Krasske) Hustedt
Navicula pupula var. nyassensis (Müller) Lange-Bertalot
Navicula radiosa Kützing
Navicula radiosafallax Lange-Bertalot
Navicula radiosiola Lange-Bertalot
Navicula recens Lange-Bertalot
Navicula regularis Hustedt
Navicula reichardtiana Lange-Bertalot
Navicula reichardtiana var. crassa Lange-Bertalot et Hofmann
Navicula reinhardtii (Grunow) Grunow
Navicula rhynchocephala Kützing
Navicula rhynchocephala var. amphiceros (Kützing) Grunow
Navicula riediana Lange-Bertalot et Rumrich
Navicula rostellata Kützing
Navicula rotunda Hustedt
Navicula rotundata Hantzsch
Navicula ruttneri var. rostrata Hustedt
Navicula salinarum Grunow
Navicula salinicola Hustedt
Navicula schadei Krasske
Navicula schmassmannii Hustedt
Navicula schroeterii Meister
Navicula schroeterii Meister
Navicula secreta var. apiculata Patrick
Navicula semen Ehrenberg
Navicula seminuloides Hustedt
Navicula splendicula VanLandingham
Navicula spp.
Navicula stankovicii Hustedt
Navicula striolata (Grunow) Lange-Bertalot in Krammer et Lange-Bertalot
Navicula stroemii Hustedt
Navicula subadnata Hustedt
Navicula subinflata Grunow
Navicula subinflatoides Hustedt
Navicula sublucidula Hustedt

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Navicula subminuscula Manguin
Navicula submuralis Hustedt
Navicula subplacentula Hustedt
Navicula subrhynchocephala Hustedt
Navicula subrhyncocephala Hustedt
Navicula subrotundata Hustedt
Navicula suecorum var. dismutica (Hustedt) Lange-Bertalot
Navicula symmetrica Patrick
Navicula tenelloides Hustedt
Navicula tridentula Krasske
Navicula tripunctata (Müller) Bory
Navicula trivialis Lange-Bertalot
Navicula trivialis var. oligotraphenta Lange-Bertalot et Hofmann
Navicula trophicatrix Lange-Bertalot in Lange-Bertalot et Metzeltin
Navicula upsaliensis (Grunow) Peragallo
Navicula utermoehlii Hustedt
Navicula vaucheriae Petersen
Navicula veneta Kützing
Navicula ventralis Krasske
Navicula viridula (Kützing) Kützing emend. Van Heurck
Navicula viridulacalcis (Hustedt) Lange-Bertalot
Navicula vitabunda Hustedt
Navicula vulpina Kützing
Navicula wiesneri Lange-Bertalot
Navicula wildii Lange-Bertalot
Navicula witkowskii Lange-Bertalot, Iserentant et Metzeltin
Naviculadicta elorantana Lange-Bertalot in Lange-Bertalot et Metzeltin
Naviculadicta raederiae Lange-Bertalot in Lange-Bertalot et Metzeltin
Neidium affine (Ehrenberg) Pfitzer
Neidium alpinum Hustedt
Neidium amphigomphus (Ehrenberg) Pfitzer
Neidium ampliatum (Ehrenberg) Krammer
Neidium apiculatum Reimer
Neidium binodeformis Krammer
Neidium binodis (Ehrenberg) Hustedt
Neidium bisulcatum (Lagerstedt) Cleve
Neidium calvum Østrup

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Neidium capsulare Thomas et Kociolek
Neidium carterii Krammer
Neidium densestriatum (Østrup) Krammer
Neidium dilatatum (Ehrenberg) Cleve
Neidium dubium (Ehrenberg) Cleve
Neidium excisum Krammer et Metzeltin
Neidium hercynicum Mayer
Neidium hitchcockii (Ehrenberg) Cleve
Neidium iridis (Ehrenberg) Cleve
Neidium iridis var. subundulatum (Cleve-Euler) Reimer
Neidium ladogensis (Cleve) Foged
Neidium productum (Smith) Cleve
Neidium septentrionale Cleve-Euler
Neidium spp.
Neidium stoermerii Thomas et Kociolek
Neidium temperei Reimer
Nitzschia acicularioides Hustedt
Nitzschia acicularis (Kützing) Smith
Nitzschia acidoclinata Lange-Bertalot
Nitzschia acula Hantzsch
Nitzschia aequorea Hustedt
Nitzschia agnita Hustedt
Nitzschia alpina Hustedt
Nitzschia amphibia Grunow
Nitzschia angusta (Gregory)
Nitzschia angustata (Smith) Grunow
Nitzschia angustatula Lange-Bertalot
Nitzschia angustiforaminata Lange-Bertalot
Nitzschia archibaldii Lange-Bertalot
Nitzschia aurariae Cholnoky
Nitzschia bacilliformis Hustedt
Nitzschia bacillum Hustedt
Nitzschia behrei Hustedt
Nitzschia biacrula Hohn et Hellerman
Nitzschia bita Hohn et Hellerman
Nitzschia bremensis Hustedt
Nitzschia brevissima Grunow ex Van Heurck

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Nitzschia bryophila (Hustedt) Hustedt
Nitzschia bulnheimiana Rabenhorst
Nitzschia calcicola Aleem et Hustedt
Nitzschia capitellata Hustedt
Nitzschia circumsuta (Bailey) Grunow
Nitzschia clausii Hantzsch
Nitzschia communis Rabenhorst
Nitzschia commutata Grunow
Nitzschia commutatoides Lange-Bertalot
Nitzschia compressa var. balatonis Lange-Bertalot
Nitzschia compressa var. minor H.L. Smith ex Boyer
Nitzschia desertorum Hustedt
Nitzschia dissipata (Kützing) Grunow
Nitzschia dissipata var. media (Hantzsch) Grunow
Nitzschia dissipata var. undulata Sovereign
Nitzschia distans Gregory
Nitzschia diversa Hustedt
Nitzschia draveillensis Coste et Ricard
Nitzschia dubia Smith
Nitzschia elegantula Grunow
Nitzschia filiformis (Smith) Van Heurck
Nitzschia flexa Schumann
Nitzschia flexoides Geitler
Nitzschia fonticola Grunow
Nitzschia fonticola var. pelagica Hustedt
Nitzschia fossilis Grunow
Nitzschia frustulum (Kützing) Grunow
Nitzschia gessneri Hustedt
Nitzschia gisela Lange-Bertalot
Nitzschia gracilis Hantzsch ex Rabenhorst
Nitzschia gracilliformis Lange-Bertalot et Simonsen
Nitzschia graciloides Hustedt
Nitzschia hantzschiana Rabenhorst
Nitzschia harderi Hustedt
Nitzschia heufleriana Grunow
Nitzschia homburgiensis Lange-Bertalot
Nitzschia hybrida Grunow

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Nitzschia hybridaeformis Hustedt
Nitzschia incognita Legler et Krasske
Nitzschia inconspicua Grunow
Nitzschia intermedia Hantzsch ex Cleve et Grunow
Nitzschia lacunarum Hustedt
Nitzschia lacuum Lange-Bertalot
Nitzschia lanceolata fo. minima Grunow
Nitzschia lanceolata Smith
Nitzschia lanceolata var. minutula Grunow
Nitzschia leistikowii Lange-Bertalot
Nitzschia levidensis var. salinarum Grunow
Nitzschia liebethruthii Rabenhorst
Nitzschia linearis (Agardh ex Smith) Smith
Nitzschia linearis var. tenuis (Smith) Grunow ex Cleve et Grunow
Nitzschia lorenziana Grunow
Nitzschia macilenta Gregory
Nitzschia microcephala Grunow
Nitzschia modesta Hustedt
Nitzschia nana Grunow ex Van Heurck
Nitzschia obtusa Smith
Nitzschia palea (Kützing) Smith
Nitzschia paleacea Grunow ex Van Heurck
Nitzschia paleaeformis Hustedt
Nitzschia parvula Smith
Nitzschia pellucida Grunow
Nitzschia perminuta (Grunow) Peragallo
Nitzschia perspicua Cholnoky
Nitzschia pseudofonticola Hustedt
Nitzschia pumila Hustedt
Nitzschia pura Hustedt
Nitzschia pusilla Grunow
Nitzschia radicula Hustedt
Nitzschia recta Hantzsch ex Rabenhorst
Nitzschia reversa Smith
Nitzschia romana Grunow
Nitzschia rosenstockii Lange-Bertalot
Nitzschia rostellata Hustedt

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Nitzschia scalpelliformis Grunow
Nitzschia sigma (Kützing) Smith
Nitzschia sigmoidea (Nitzsch) Ehrenberg
Nitzschia silicula Hustedt
Nitzschia siliqua Archibald
Nitzschia sinuata (Thwaites) Grunow
Nitzschia sinuata var. delognei (Grunow) Lange-Bertalot
Nitzschia sinuata var. tabellaria (Grunow) Grunow
Nitzschia sociabilis Hustedt
Nitzschia solita Hustedt
Nitzschia spp.
Nitzschia subacicularis Hustedt
Nitzschia subconstricta Grunow
Nitzschia sublinearis Hustedt
Nitzschia subrostrata Hustedt
Nitzschia subtilioides Hustedt
Nitzschia subtilis Grunow
Nitzschia suchlandtii Hustedt
Nitzschia supralitorea Lange-Bertalot
Nitzschia terrestris (Petersen) Hustedt
Nitzschia thermaloides Hustedt
Nitzschia tropica Hustedt
Nitzschia tubicola Grunow in Cleve et Grunow
Nitzschia umbonata Lange-Bertalot
Nitzschia valdecostata Lange-Bertalot et Simonsen
Nitzschia valdestriata Aleem et Hustedt
Nitzschia vermicularis (Kützing) Hantzsch in Rabenhorst
Nitzschia vitrea Norman
Nitzschia vitrea var. salinarum Grunow
Nitzschia wuellerstorffii Lange-Bertalot
Nupela carolina Potapova et Clason
Nupela fennica (Hustedt) Lange-Bertalot
Nupela impexiformis (Lange-Bertalot) Lange-Bertalot
Nupela lapidosa (Krasske) Lange-Bertalot
Nupela neglecta Ponader, Lowe et Potapova
Nupela neotropica Lange-Bertalot
Nupela spp.

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Nupela vitiosa (Schimanski) Siver et Hamilton
Nupela wellneri (Lange-Bertalot) Lange-Bertalot
Opephora martyi Héribaud
Opephora olsenii Møller
Opephora schwartzii (Grunow) Petit
Orthoseira dentroteres (Ehrenberg) Crawford
Orthoseira roeseana (Rabenhorst) O'Meara
Parlibellus crucicula (Smith) Witkowski, Lange-Bertalot et Metzeltin
Parlibellus protracta (Grunow) Witkowski, Lange-Bertalot et Metzeltin
Peronia fibula (Brébisson ex Kützing) Ross
Pinnuavis elegans (Smith) Okuno
Pinnularia abaujensis (Pantocsek) Ross
Pinnularia acidophila Hofmann et Krammer
Pinnularia acoricola Hustedt
Pinnularia acrosphaeria (Brébisson) Smith
Pinnularia acrosphaeria var. turgidula Grunow ex Cleve
Pinnularia aestuarii Cleve
Pinnularia angusta (Cleve) Krammer
Pinnularia appendiculata (Agardh) Cleve
Pinnularia biceps Gregory
Pinnularia biceps var. pusilla Charles et Camburn
Pinnularia bihastata (Mann) Patrick
Pinnularia borealis Ehrenberg
Pinnularia borealis var. rectangularis Carlson
Pinnularia borealis var. scalaris (Ehrenberg) Rabenhorst
Pinnularia brandelii Cleve
Pinnularia braunii (Grunow) Cleve
Pinnularia braunii var. amphicephala fo. subconica Venkataraman
Pinnularia brebissonii (Kützing) Rabenhorst
Pinnularia brevicostata Cleve
Pinnularia cleveii Patrick
Pinnularia dactylus Ehrenberg
Pinnularia divergens Smith
Pinnularia divergentissima (Grunow) Cleve
Pinnularia divergentissima var. subrostrata Cleve-Euler
Pinnularia formica (Ehrenberg) Patrick
Pinnularia gibba Ehrenberg

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Pinnularia gigas Ehrenberg
Pinnularia globiceps Gregory
Pinnularia hemiptera (Kützing) Rabenhorst
Pinnularia hemiptera var. bielawskii (Héribaud et M. Peragallo in Héribaud) Cleve-Euler
Pinnularia hilseana Janisch
Pinnularia ignobilis (Krasske) Cleve-Euler
Pinnularia intermedia (Lagerstedt) Cleve
Pinnularia interrupta Smith
Pinnularia krammeri Metzeltin in Lange-Bertalot et Metzeltin
Pinnularia lagerstedtii (Cleve) Cleve-Euler
Pinnularia lapponica Hustedt
Pinnularia legumen (Ehrenberg) Ehrenberg
Pinnularia lundii Hustedt
Pinnularia macilenta (Ehrenberg) Ehrenberg
Pinnularia maior (Kützing) Rabenhorst
Pinnularia mesogongyla Ehrenberg
Pinnularia mesolepta (Ehrenberg) Smith
Pinnularia mesolepta (Ehrenberg) Smith
Pinnularia microstauron (Ehrenberg) Cleve
Pinnularia molaris (Grunow) Cleve
Pinnularia nobilis (Ehrenberg) Ehrenberg
Pinnularia nodosa (Ehrenberg) Smith
Pinnularia nodosa var. percapitata Krammer
Pinnularia obscura Krasske
Pinnularia paulensis Grunow ex Cleve
Pinnularia pogoii Scherer
Pinnularia polyonca (Brébisson) Smith
Pinnularia renata Krammer
Pinnularia rupestris Hantzsch
Pinnularia similis Hustedt
Pinnularia sinistra Krammer
Pinnularia sistassa Carter
Pinnularia spp.
Pinnularia stomatophora (Grunow) Cleve
Pinnularia streptoraphe Cleve
Pinnularia subcapitata Gregory
Pinnularia subcommutata var. nonfasciata Krammer

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Pinnularia subgibba Krammer
Pinnularia sublinearis Grunow
Pinnularia subrostrata (Cleve) Cleve-Euler
Pinnularia sudetica (Hilse) Peragallo
Pinnularia superdivergentissima Chaumont et Germain
Pinnularia umbrosa Sovereign
Pinnularia viridiformis Krammer
Pinnularia viridis (Nitzsch) Ehrenberg
Placoneis abiskoensis (Hustedt) Lange-Bertalot et Metzeltin
Placoneis anglica (Ralfs in Pritchard) R. Lowe in J.R. Johansen et al.
Placoneis clementis (Grunow) Cox
Placoneis clementoides (Hustedt) Cox
Placoneis elginensis (Gregory) Cox
Placoneis exigua (Gregory) Mereschkowsky
Placoneis explanata (Hustedt) Cox
Placoneis gastrum (Ehrenberg) Mereschkowsky
Placoneis pseudanglica (Lange-Bertalot) Cox
Placoneis spp.
Plagiotropis lepidoptera (Gregory) Kuntze
Plagiotropis lepidoptera var. proboscidea (Cleve) Reimer
Plagiotropis vitrea (Smith) Cleve et Möller
Planothidium apiculatum (Patrick) Lange-Bertalot
Planothidium biporomum (Hohn et Hellerman) Lange-Bertalot
Planothidium calcar (Cleve) Bukhtiyarova et Round
Planothidium daui (Foged) Lange-Bertalot
Planothidium delicatulum (Kützing) Round et Bukhtiyarova
Planothidium distinctum (Messikommer) Lange-Bertalot
Planothidium fossile (Tempère et Peragallo) Lowe
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot
Planothidium hauckianum (Grunow) Round et Bukhtiyarova
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot
Planothidium joursacense (Héribaud) Lange-Bertalot
Planothidium lanceolatum (Brébisson) Lange-Bertalot
Planothidium minutissimum (Krasske) Lange-Bertalot
Planothidium oestrupii (Cleve-Euler) Round et Bukhtiyarova
Planothidium peragalli Brun et Héribaud
Planothidium peragalli var. parvulum (Patrick) Andresen, Stoermer et Kreis

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Planothidium robustius (Hustedt) Lange-Bertalot
Planothidium spp.
Planothidium stewartii (Patrick) Lange-Bertalot
Planothidium ziegleri (Lange-Bertalot) Lange-Bertalot
Platessa conspicua (Mayer) Lange-Bertalot
Platessa holsatica (Hustedt) Lange-Bertalot
Platessa hustedtii (Krasske) Lange-Bertalot
Pleurosigma angulatum (Quekett) Smith
Pleurosigma delicatulum Smith
Pleurosigma elongatum Smith
Pleurosigma salinarum Grunow
Pleurosigma spp.
Pleurosira laevis (Ehrenberg) Compère
Psammodictyon constrictum (Gregory) Mann
Psammodictyon panduriforme (Gregory) Mann
Psammothidium abundans fo. rosenstockii Lange-Bertalot ex Lange-Bertalot
Psammothidium altaicum Bukhtiyarova
Psammothidium bioretii (Germain) Bukhtiyarova et Round
Psammothidium chlidanos (Hohn et Hellerman) Lange-Bertalot
Psammothidium didymum (Hustedt) Bukhtiyarova et Round
Psammothidium grischunum (Wuthrich) Bukhtiyarova et Round
Psammothidium helveticum (Hustedt) Bukhtiyarova et Round
Psammothidium kryophilum (Petersen) Reichardt
Psammothidium kuelbsii (Lange-Bertalot et Krammer) Bukhtiyarova et Round
Psammothidium lacus-vulcani (Lange-Bertalot et Krammer) Bukhtiyarova et Round
Psammothidium lauenburgianum (Hustedt) Bukhtiyarova et Round
Psammothidium levanderi (Hustedt) Czarnecki
Psammothidium marginulatum (Grunow) Bukhtiyarova et Round
Psammothidium rossii (Hustedt) Bukhtiyarova et Round
Psammothidium sacculum (Carter) Bukhtiyarova
Psammothidium scoticum (Flower et Jones) Bukhtiyarova et Round
Psammothidium spp.
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round
Psammothidium ventralis (Krasske) Bukhtiyarova et Round
Pseudostaurosira brevistriata (Grunow) Williams et Round
Pseudostaurosira clavatum Morales
Pseudostaurosira parasitica (Smith) Morales

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Pseudostaurosira parasitica var. subconstricta (Grunow) Morales
Pseudostaurosira polonica Morales et Edlund
Pseudostaurosira pseudoconstruens (Marciniak) Williams et Round
Pseudostaurosira robusta (Fusey) Williams et Round
Pseudostaurosira spp.
Pseudostaurosira subsalina (Hustedt) Morales
Pseudostaurosira trainorii Morales
Pseudostaurosira zeilleri (Héribaud) Williams et Round
Pseudostaurosiropsis spp.
Pseudostaurosiropsis spp.
Puncticulata bodanica (Grunow in Schneider) Håkansson
Puncticulata comta (Ehrenberg) Håkansson
Puncticulata radiosa (Lemmermann) Håkansson
Puncticulata spp.
Reimeria sinuata (Gregory) Kociolek et Stoermer
Reimeria uniseriata Sala, Guerrero et Ferrario
Rhizosolenia eriensis Smith
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot
Rhoicosphenia spp.
Rhopalodia acuminata Krammer
Rhopalodia brebissonii Krammer
Rhopalodia gibba (Ehrenberg) Müller
Rhopalodia gibba var. minuta Krammer
Rhopalodia gibberula (Ehrenberg) Müller
Rhopalodia musculus (Kützing) Müller
Rhopalodia operculata (Agardh) Håkansson
Rhopalodia rupestris (Smith) Krammer
Rhopalodia spp.
Rossithidium duthii (Sreenivasa) Kingston
Rossithidium linearis (Smith) Round et Bukhtiyarova
Rossithidium petersennii (Hustedt) Round et Bukhtiyarova
Rossithidium pusillum (Grunow) Round et Bukhtiyarova
Scoliotropis latestriata (Brébisson in Kützing) Cleve
Sellaphora alastos (Hohn et Hellerman) Lange-Bertalot et Metzeltin
Sellaphora americana (Ehrenberg) Mann
Sellaphora bacillum (Ehrenberg) Mann
Sellaphora disjuncta (Hustedt) Mann

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Sellaphora hustedtii (Krasske) Lange-Bertalot
Sellaphora laevissima (Kützing) Mann
Sellaphora mutata (Krasske) Lange-Bertalot
Sellaphora pupula (Kützing) Meresckowsky
Sellaphora seminulum (Grunow) Mann
Sellaphora spp.
Simonsenia delognei (Grunow) Lange-Bertalot
Skeletonema costatum (Greville) Cleve
Skeletonema potamos (Weber) Hasle
Stauroforma exiguiformis (Lange-Bertalot) Flower, Jones et Round
Stauroneis acuta Smith
Stauroneis agrestis Petersen
Stauroneis alpina Hustedt
Stauroneis anceps Ehrenberg
Stauroneis borrichii (Petersen) Lund
Stauroneis dubitabilis Hustedt
Stauroneis fluminea Patrick et Freese
Stauroneis gracilis Ehrenberg
Stauroneis gracillima Hustedt
Stauroneis kriegeri Patrick
Stauroneis legumen (Ehrenberg) Kützing
Stauroneis livingstonii Reimer
Stauroneis nebulosa (Krasske) Lange-Bertalot in Lange-Bertalot, Kulbs, Lauser, Norpel-Schempp et Willmann
Stauroneis neohyalina Lange-Bertalot et Krammer in Lange-Bertalot et Metzeltin
Stauroneis nobilis Schumann
Stauroneis obtusa Lagerstedt
Stauroneis phoenicenteron (Nitzsch) Ehrenberg
Stauroneis phoenicenteron (Nitzsch) Ehrenberg
Stauroneis producta Grunow
Stauroneis prominula (Grunow) Hustedt
Stauroneis recondita Krasske
Stauroneis schimanskii Krammer
Stauroneis siberica (Grunow in Cleve et Grunow) Lange-Bertalot et Krammer in Lange-Bertalot et Metzeltin
Stauroneis smithii Grunow
Stauroneis spp.

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Stauroneis staurolineata Reimer
Stauroneis stodderi Lewis
Stauroneis stodderi Lewis
Stauroneis tackei Krammer et Lange-Bertalot
Stauroneis thermicola (Petersen) Lund
Staurosira bidens (Heiberg) Grunow
Staurosira construens Ehrenberg
Staurosira construens var. venter (Ehrenberg) Hamilton
Staurosira spp.
Staurosirella berolinensis (Lemmermann) Bukhtiyarova
Staurosirella lapponica (Grunow) Williams et Round
Staurosirella leptostauron (Ehrenberg) Williams et Round
Staurosirella oldenburgiana (Hustedt) Morales
Staurosirella pinnata (Ehrenberg) Williams et Round
Staurosirella spp.
Staurosirella subrobusta Morales et Manoylov
Stenopterobia anceps (Lewis) Brébisson ex Van Heurck
Stenopterobia curvula (Smith) Krammer
Stenopterobia delicatissma (Lewis) Brébisson
Stenopterobia densestriata (Hustedt) Krammer
Stenopterobia planctonica Metzeltin et Lange-Bertalot
Stenopterobia spp.
Stephanodiscus agassizensis Håkansson et Kling
Stephanodiscus agassizensis Håkansson et Kling
Stephanodiscus alpinus Hustedt
Stephanodiscus astraea (Ehrenberg) Grunow
Stephanodiscus binderanus (Kützing) Krieger
Stephanodiscus binderanus var. oestrupii Cleve-Euler
Stephanodiscus carconensis Grunow
Stephanodiscus excentricus Hustedt emend Håkansson et Stoermer
Stephanodiscus hantzschii Grunow
Stephanodiscus medius Håkansson
Stephanodiscus minutulus (Kützing) Cleve et Möller
Stephanodiscus neoastraea Håkansson et Hickel
Stephanodiscus niagarae Ehrenberg
Stephanodiscus rotula (Kützing) Hendey
Stephanodiscus spp.

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Stephanodiscus subtranssylvanicus Gasse
Stephanodiscus vestibulis Håkansson, Theriot et Stoermer
Stephanodiscus yellowstonensis Theriot et Stoermer
Surirella amphioxys Smith
Surirella angusta Kützing
Surirella bifrons Ehrenberg
Surirella birostrata Mayer
Surirella biseriata Brébisson
Surirella bohemica Maly
Surirella brebissonii Krammer et Lange-Bertalot
Surirella brebissonii var. kuetzingii Krammer et Lange-Bertalot
Surirella brebissonii var. punctata Krammer
Surirella brightwellii Smith
Surirella capronii Brébisson
Surirella constricta Smith
Surirella crumena Brébisson
Surirella didyma Kützing
Surirella elegans Ehrenberg
Surirella gracilis (Smith) Grunow
Surirella guatimalensis Ehrenberg
Surirella helvetica Brun
Surirella helvetica Brun
Surirella hoefleri Hustedt
Surirella linearis Smith
Surirella linearis var. constricta Grunow
Surirella minuta Brébisson
Surirella minuta Brébisson
Surirella ovalis Brébisson
Surirella ovalis Brébisson
Surirella patella Kützing
Surirella peisonis Pantocsek
Surirella roba Leclercq
Surirella robusta Ehrenberg
Surirella splendida (Ehrenberg) Kützing
Surirella spp.
Surirella stalagma Hohn et Hellerman
Surirella striatula Turpin

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Surirella subsalsa var. minuta Smith
Surirella tenera Gregory
Surirella tenuis Mayer
Surirella utahensis (Grunow in Schmidt et al.) Hanna et Grant
Surirella venusta Østrup
Surirella visurgis Hustedt
Synedra acus Kützing
Synedra acus Kützing
Synedra biceps Kützing
Synedra capitata Ehrenberg
Synedra cyclopum Brutschy
Synedra delicatissima Smith
Synedra delicatissima Smith
Synedra demerarae Grunow
Synedra dilatata Brébisson
Synedra filiformis Grunow
Synedra goulardi Brébisson
Synedra incisa Boyer
Synedra mazamaensis Sovereign
Synedra rumpens Kützing
Synedra rumpens Kützing
Synedra socia Wallace
Synedra spp.
Synedra ulna (Nitzsch) Ehrenberg
Tabellaria binalis (Ehrenberg) Grunow
Tabellaria binalis var. elliptica Flower
Tabellaria fenestrata (Lyngbye) Kützing
Tabellaria flocculosa (Roth) Kützing
Tabellaria flocculosa var. linearis Koppen
Tabellaria quadriseptata Knudsen
Tabellaria spp.
Tabellaria ventricosa Kützing
Tabularia fasciculata (Agardh) Williams et Round
Tabularia tabulata (Agardh) Snoeijs
Tetracyclus glans (Ehrenberg) Mills
Tetracyclus spp.
Thalassionema nitzschioides (Grunow) Van Heurck

NLA Taxa
NLA Diatom and Non-Diatom Taxa
Thalassiosira baltica (Grunow) Ostenfeld
Thalassiosira bramaputrae (Ehrenberg) Håkansson et Locker
Thalassiosira oestrupii (Ostenfeld) Hasle
Thalassiosira proschkinae Makarova
Thalassiosira pseudonana Hasle et Heimdal
Thalassiosira spp.
Thalassiosira tenera Proshkina-Lavrenko
Thalassiosira visurgis Hustedt
Thalassiosira visurgis Hustedt
Thalassiosira weissflogii (Grunow) Fryxell et Hasle
Tryblionella acuminata Smith
Tryblionella aerophila (Hustedt) Mann
Tryblionella calida (Grunow in Cleve et Grunow) Mann
Tryblionella coarctata (Grunow) Mann
Tryblionella compressa (Bailey) Poulin
Tryblionella constricta (Kützing) Ralfs
Tryblionella debilis Arnott
Tryblionella gracilis Smith
Tryblionella hungarica (Grunow) Mann
Tryblionella levidensis Smith
Tryblionella levidensis Smith
Tryblionella littoralis (Grunow) Mann
Tryblionella peisonis (Pantocsek) F.W. Mills
Tryblionella plana (Smith) Pelletan
Tryblionella scalaris (Ehrenberg) Siver et Hamilton
Tryblionella spp.
Tryblionella umbilicata (Hustedt) Mann
Tryblionella victoriae Grunow
Urosolenia longiseta (Zacharias) Bukhtiyarova

Appendix C

Supplementary Material for Vegetation—Lists of Floristic Resources

Region I

Ballard, B.D, H.L. Whittier, C.A. Nowark. 2004. Northeastern Shrub and Short Tree Identification. SUNY. 120 pp.

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Common Wetland Delineation Sedges of the Northeast - Robert Lichvar

Cope. 2001. *Muenscher's Keys to Woody Plants. An Expanded Guide to Native and Cultivated Species.* Comstock Publishing Associates. Cornell University Press. Ithaca. 368 pp.

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Gleason, H.A., N.H. and P.K. Holmgren. 1998. Illustrated Companion to Gleason and Cronquit's Manual: Illustrations of the Vascular Plants of Northeastern United States and Adjacent Canada. New York Botanical Garden, New York. 937 pp.

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Gleason, H.A., N.H. and P.K. Holmgren. 1998. Illustrated Companion to Gleason and Cronquit's Manual: Illustrations of the Vascular Plants of Northeastern United States and Adjacent Canada. New York Botanical Garden, New York. 937pp.

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Strausbaugh, P. D. and E. L Core. 1978. Flora of West Virginia, Second Edition. Seneca Books, Morgantown. 1079 pp.

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Smith. E.B. 1994. Keys to the Flora of Arkansas. University of Arkansas Press. 376 pp.

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Parish, R. 1999. Plants of Southern Interior British Columbia and the Inland Northwest. Lone Pine Publishing 464 pp.

Tuner, M. and P. Gustafson. 2006. Wildflowers of the Pacific Northwest. Timber Press, Inc 512 pp.

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http://info.ag.uidaho.edu/pdf/SB/SB039.pdf

Appendix D

Plant Pressing and Mounting

Pressing Samples

Plants must be pressed and dried immediately upon reception at the. Immediately pressing and drying results in the highest quality samples which facilitate identification.

Standard Procedures for Pressing Plant Samples

Samples are pressed and dried in a standard plant press (30 X 45 cm or 12 X 18 inches), composed of:

a. A rigid, breathable wooden frame
b. Corrugated cardboard ventilators to allow air flow through the press
c. Blotter paper to absorb moisture
d. Folded newsprint to contain the plant material
e. Straps with buckles to tighten the press

Assembling the press

Each newspaper sample folder with plant material is sandwiched between 2 moisture-absorbing blotters. The "blotter-newsprint sandwiches" are sandwiched between corrugated cardboard, ensuring that the corrugations of the cardboard run parallel to the shorter dimension (30 cm) for best air circulation. Bulky samples may require extra blotters and cardboard. Specifics on how to display plant material within the newsprint folders and assemble the press are provided below:

7. On the bottom wooden frame of the press, place cardboard and on top the cardboard add a blotter. Lay a newsprint folder on top of the blotter.
8. Plant material will be placed inside this sheet of folded newsprint (folded size 30 X 45 cm). Ideally, samples should not exceed this size so that they can be entirely enclosed in the plant press and because the heavy herbarium paper on which samples may later be mounted has dimensions of 29 X 42 cm (11.5 x 16.5 inches).
9. For every plant sample, clearly write on the outside of its newsprint folder, using water-proof ink, the sample number (see Vegetation Chapter in FOM) and other pertinent tracking information (plant name or pseudonym, X-Point number, collection date, and collector(s) name(s)).
10. Clean as much dirt as possible off the plant material before placing it in the newsprint folder.
11. Open the newsprint folder and place a filled out Plant Sample Label Form (see Fig. 4-3) on the bottom fold of the paper.
12. Carefully arrange the plant material on the bottom fold of the newsprint to display diagnostic features.
13. Lay the plants flat and avoid overlapping plant parts.
14. Spread leaves, flowers, and fruits so they can be easily observed from different perspectives.
15. Show upper and lower surfaces of leaves and flowers.

16. If possible, arrange pressed material to show some flowers with the blossom open, and some flowers and fruits are pressed in longitudinal and transverse views.
17. Multiples of smaller plants should be pressed together in order to provide ample material for study.
18. For large samples, bend stems sharply into a V, N or W shapes so they fit within the press frame. Do not curve or twist the stems.
19. In order to insure rapid and thorough drying, thick stems, large fruits, or bulbs may be cut in half lengthwise. Areas around thick structure may need additional absorptive layers, e.g., extra newspaper or blotters.
20. Examples of small, loose plant parts, such as seeds or Carex perigynia, should be placed in a small paper packet or envelope inside of the newspaper.
21. Once the plant material is satisfactorily arranged, fold the newsprint closed.
22. Add another blotter, then a cardboard on top of the newsprint folder. To begin pressing the next sample, place a blotter over the top cardboard in the stack. Add a newsprint folder and repeat steps 3 – 13 until the press is full or all samples are included.
23. Use two adjustable straps to firmly hold the plant press and its contents. The plant press must be kept tight to prevent shrinkage and wrinkling of the plant material. It should not be possible to move the blotters or cardboards from the side in a properly tightened press.

Mounting Plant Samples

Vascular plants should:

- Be mounted on archival-quality paper measuring 11.5 X 16.5 inches
- Be mounted using commercially available acid free adhesive, such as polyvinyl acetate (PVA)
- Allow for placement of a properly filled out label
- Have an acid free fragment envelop (where necessary for seeds and flowers)

Labeling Herbarium Sheets

Herbarium labels are considered field data, and an herbarium sheet is incomplete in the absence of accurate label data. A completed Plant Sample Label Form (Figure 4-5) should be affixed to the herbarium sheet in preparation for storage. Much of the information required to complete the label Data Form is available on the Plant Sample Label Form (Vegetation Chapters in FOM) EPA will provide Label Data Forms.

Information to be included on voucher sample labels

- **Sample Number:** NWCA Site Number-Plant sample number-Plot module number (if applicable). Plant sample numbers for Samples are ordered chronologically for each site beginning with one. For example, the Sample number for the 5th species collected at NWCA X-Point 0762 in Plot module 3 would be NWCA-0762-005-3. For Samples not collected in a vegetation plot, the plot module number is 0.
- **Scientific name**: The Genus, species, and authority are recorded.
- **Collector(s) name**: List the first name, middle initial, and surname of the person or persons who collected the Sample.
- **Date of collection**: List the day, month (spelled out or abbreviated), and 4 digit year, e.g., 3 May 2003, for when the collection was made.
- **Identified By:** This item is left blank until the Sample is identified. Upon identification, the first name, middle initial, and surname of the person who identified the plant.
- **Verified By:** This item is filled in only for cases where a second person verifies the first identification, or corrects the original identification. The first, middle initial, and surname of the person who did the verification or made the correction. If a name correction is made, it is noted in the Scientific Name field and initialed and dated by the verifying botanist.
- **Detailed location:** (note: much of this information should be available in site packets or on other data forms – so perhaps need not be repeated here as long as it is available to whoever is doing plant identification or to herbaria where vouchers might be deposited):
    - County and state
    - Public or private land and land manager or landowner information
    - Description of the location in reference to roads, road junctions, mile markers and distances from cities and/or towns, or nearby natural features.
    - Latitude and longitude, section, township and range
    - Global Positioning System (GPS) coordinates
    - Elevation
- **Habitat**: The type of plant community or setting where the plant is growing. (e.g., such as wetland type (Cowardin, NVC, HGM), wetland community type (forested wetland,

emergent marsh, wet prairie, mountain bog, etc.), anthropogenic disturbances (urban setting type), and, if known, other plants growing in association (associated species information would be available from the plot.

- **Plant habit**: Describe key features of the plant such as growth form (tree, shrub, vine, herb), approximate height, longevity (annual, biennial, perennial), clonal, rhizomatous, tussock-forming, etc.
- **Frequency**: Indicate whether the species is rare, occasional, frequent or common at the site, and whether based on canopy cover it is a dominant, moderate cover, or low cover species.
- **Plant description**: List any characteristics of the plant which may be lost upon drying, such as flower/fruit color and fragrance, leaf orientation and aroma.

Plants of the National Wetlands Condition Assessment

Species Name Family Name

Detailed Location:

Habitat:

Plant Habit:

Plant Description:

Collector's Name: Specimen Number Date (Month, Day, Year)

Identifier's name

Figure 4-5: Format for Herbaria Labels