Lab Protocols for the Testing of Eastern Deciduous Forest Soils

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Preface

Purpose
The purpose of this document is threefold: (1) to provide a clear and precise set of laboratory procedures for the routine processing and analysis of forest soils, (2) to provide a baseline set of procedures that can be easily replicated and used for comparative purposes when conducting meta-analyses, (3) to provide a starting point for Department of Environmental and Plant Biology students of Ohio University (or elsewhere) wishing to do their own soil analysis.

Focus
These protocols are not intended to be comprehensive. The document is designed to address the major physical and chemical attributes of forest soils that may affect plant distribution and abundance. There are many fine, comprehensive procedural manuals dedicated to the physical and chemical analysis of soils (see reference list at the end of this document). Here, I have selected and summarized what I feel to be relatively standard procedures from a variety of sources.

My intent is to provide field & laboratory procedures that (1) are relatively easy to follow, (2) do not require inordinately expensive lab equipment or supplies, (3) that are easily accessible (i.e., make few assumptions about chemistry or laboratory
background), and (4) are easy to replicate and reasonably accurate.

Due to the vast amount of research in soil science, virtually every procedure supplied here is arguable (every procedure has its strengths and weaknesses); but in my experience, these procedures have proven to be reasonably reliable (within 5%) when compared to blind side tests done at independent soil testing laboratories.

**Abbreviations**

A variety of abbreviations have been used throughout this document to conserve space. I have employed SI units for all measures of mass, length, temperature, time and include a few less standard abbreviations.

- ca. (*circa* = approximately)
- µg (micrograms)
- PSI (pounds per square inch)
- NB (*nota bene* = note well)
- mg (milligrams)
- OPM (oscillations per minutes)
- C (degrees centigrade)
- g (grams)
- T (transmission)
- min (minutes)
- nm (nanometers)
- A (absorbance)
- h (hours)
- mm (millimeters)
- ® (registered trade mark)
- wk (week)
- cm (centimeters)
- # (number; size, or part)
- ppm (parts per million)
- m (meters)
- dH₂O (distilled water)
- qt (quart)
- ddH₂O (distilled & deionized water)
Soil Collection Procedures

- Remove all organic horizons just to the surface of bare mineral soil.
- Use a tube or bulb sampler (or trowel if rocky) to sample top 10 cm of A-horizon.
- Take 2-3 samples within a defined area of ca. 0.5 m².
- Make sure you collect at least 250 g of soil total.
- Transfer to marked, zip-loc bags, mix, and return to lab ASAP.
- During transport, do not expose to excessive heat or cold (store in a cooler).

Supplies needed:

- Quart size zip-loc bags (heavy freezer weight)
- Sharpie marker pen

Materials needed:

- 50 qt Coleman cooler
- Oakley tube sampler
- Bulb planter
- Hand-trowel

Lab Handling and Preparation

- Store field-moist soil at 5 C (refrigerator) for no more than 72 h.
- Set aside at least 75 g of "field-moist" soil for soil moisture analysis (see below).
- Set aside at least 25 g of "field-moist" soil for soil nitrate analysis (see below).
- Place the 100 g of "field-moist" soil back in the labeled field collection bags.
- Remaining soil is spread out on 2-3 sheets thick of newspaper to dry.
- Discard any macroscopic organic debris or large stones.
- Pulverize any clods of soil on the paper (hammer or roller), air-dry at 20 C for 48 h.
- After air-drying, grind soils through a 2 mm brass sieve (discard larger remains).
- Place the dry sieved-soil into paper lunch bags or lidded paper cups & label.
- Store at room temp. (sieved & dried soil can be stored for long periods of time).
- All subsequent references to "air-dried soil" will be to the air-dried & sieved soil here.
- (NB: you should now have 2 bags for each soil sample; 1 moist, 1 dry.)

Supplies needed:

- Quart size zip-loc bags (heavy freezer weight)
- Paper lunch bags or paper cups with lids.
- Re-cycled newspaper
- Sharpie marker pen

Materials needed:

- Brass Sieve, 2 mm
- Lab balance (0.01 g)

**Nitrate Nitrogen (cadmium reduction method)**

This test should be performed as soon as is practical with field-moist soil.

Significant changes in nitrate can occur with storage of only 1 wk.

- Add 25 g of field-moist soil to a 250 ml Erlenmeyer flask.
- Add 75 ml of 0.1 M CaSO4 (1.361 g in 1000 ml) to the flask.
- Agitate on orbital flatbed shaker at 150 OPM for 15 min.
- Set flasks to stand for 1 h at 20 C.
- Gravity filter the suspension through Whatman #542 filter paper into a 125 ml flask.
- Use a long-stemmed funnel in the flask, pleat the filter paper for good feed rate.
• This step may require up to 1 hr for complete filtration.
• While you are waiting during the extraction/filtration period...

• Locate the spectrophotometer (Spec-20D), check for the appropriate bulb.
• Allow 20 min for the instrument to warm up, set the Spec-20D to 500 nm.
• Standardize to 100%T (0%A) using distilled water in a spectube.
  Continue with analysis...

• Measure out 25 ml of filtrate using a volumetric flask.
• Transfer 25 ml filtrate to a 25 x 150 mm borosilicate glass culture tube.
• Add one Nitraver-V Powder Pillow to the 25 ml of filtrate.
• Stopper the tube w/a #4 rubber stopper and shake/oscillate vigorously for 60 sec.
• Allow tube to stand in rack for ca. 10 min (an amber color should begin to develop).
• CAUTION: at this stage, the colorimetric reaction has begun; can not stand > 20 min!
• Fill a Spec-20 sample tube almost to the top with the cadmium-treated amber solution.
• Read T/A of treated sample & record.
• Construct a standard curve using known nitrate standard solutions (Hach 15 ppm).
• Construct a dilution series using 15.0, 7.5, 3.75, and 1.875 ppm for the std curve.
• Determine NO₃ concentration via standard curve.

Supplies Needed:

• Whatman #542 (125 mm) filter paper (Fisher #09-852H)
• Borosilicate glass culture tubes, 25 x 150 mm (Fisher #14-961-34)
• Nitraver-V powder pillows (Hach #14034-99)
• Nitrate standard solution, 15 ppm (Hach #24151-32) (1000 ppm standards available from Fisher.)
Materials needed:

- Erlenmeyer flasks, 250 ml
- Orbital flatbed shaker
- Long-stemmed glass funnels
- Milton-Roy Spectronic 20D spectrophotometer
- Size #4 black rubber stoppers
- Electric test-tube agitator
- Spec-20 sample tubes & racks (yellow)
- Racks for 25 mm culture tubes (blue)
- Volumetric flasks (25 ml, 50 ml, 100 ml)

**Soil Moisture (gravimetric method)**

This test should be performed as soon as is practical with field-moist soil.

Field soil can be stored in plastic at 5°C for up to 24-72 h prior to analysis.

- Obtain soil tins and fill ca. 3/4 full (75 g soil set aside from above) of fresh field-moist soil.
  
The exact amount of soil is not that important (as long as tin is > 50% full).
- Place a lid on the tin (note: lid + tin = tare).
- Weigh the soil-filled tare on a digital balance to the nearest 0.1 g and record weight.
- Place tin in an oven, with the lid slid off to the side, at 105°C for 24 h.
- Reweight the soil-filled tare and record weight.
- Empty the soil completely and weigh the tare (tin + lid).
- Determine the percent soil moisture:

\[
\%_{\text{dw}} = \frac{(\text{weight of wet soil + tare}) - (\text{weight of dry soil + tare})}{(\text{weight of dry soil + tare}) - (\text{tare})} \times 100
\]
Soil pH (glass electrode method)

Troubleshoot any pH meter problems prior to analysis.

Refill electrodes if necessary (KCl/AgCl) & test meter.

- Standardize pH meter with pH 4.0 and 7.0 buffer solution.
- Weigh 25 g of air-dried soil, and place into a 100 ml beaker.
- Add 25 ml of distilled and de-ionized water to the beaker.
- Agitate beaker on an orbital flatbed shaker at 150 OPM for 60 sec.
- Let beaker stand for 10 min.
- Insert the pH electrode into the soil-water solution and swirl gently.
- Read the pH immediately on a standardized pH meter.
- Rinse the electrode with de-ionized water & wipe lightly with a kim-wipe.

Supplies needed:

- Buffer solution pH 4.00 (Fisher #SB101-500)
- Buffer solution pH 7.00 (Fisher #SB107-500)
- de-ionized distilled water (5th floor Porter)

Materials needed:

- Corning 350 pH/ion meter w/pH electrode
- 100 ml beakers
- Orbital flatbed shaker
Organic Matter and Ash Content (dry-ash method)

- Weigh out approximately 3.0 g of air-dried and sieved soil on a lab balance.
- Dry soil at 105°C for 24 h.
- Oven dry (105°C) a high-form 30 ml porcelain crucible for 1 h.
- Weigh out 2.0 g of oven-dry soil into an oven dry crucible.
- Weigh and record mass (to nearest 0.0001 g) of the oven-dry crucible/soil sample.
- Place crucibles into a muffle furnace; bring temp to 375°C & hold for 1 h.
- Ash the sample at 600°C for 6 h.
- Allow oven to cool to 150°C, replace exact porcelain lid on crucible.
- Note: it is important to keep track of which lid goes with which crucible!
- Move warm crucibles to a dessicator filled with drierite dessicant. (NB: use tongs and hot gloves.)
- Weigh crucible + ash (to nearest 0.0001 g).
- Calculate the ash content as:

\[ Ash(\%) = \left( \frac{\text{final mass of crucible and ash} - \text{weight of empty crucible}}{\text{mass of crucible and soil sample} - \text{weight of empty crucible}} \right) \times 100 \]

\[ \text{Organic matter (\%) = 100 - Ash(\%)} \]

Supplies:
- None

Materials:
- Drying oven
- Muffle furnace
- Porcelain crucibles
- Dessicator jar
- Drierite
- Lab balance
Soil Texture (hydrometer method)

If possible, this entire procedure should be done in a constant temp room.

(NB: this procedure is not recommended for calcareous soils, saline soils, or soils > 2% organic matter.)

A. Calibrate each soil hydrometer (ASTM 152H).

- Make a Calgon® solution (sodium-hexametaphosphate) by adding 50 g to one liter.
- Add 100 ml of Calgon® solution to a glass cylinder; adjust vol up to 1 liter w/dH₂O.
- Mix thoroughly and allow to stand (ca. 30 min) at a constant temp (20-25 C).
- Lower the hydrometer gently into the solution.
- Determine the scale reading (R_L) at the upper edge of the meniscus on the stem.

B. Second, begin your soil texture analysis.

- Weigh 40.0 g of air-dried (105 C, 24 hr) & sieved soil into a 600 ml beaker.
  (Note: if the soil is a loamy sand or sandy, use 100 g of soil sample.)
- Add 100 ml of Calgon® solution and 300 ml dH₂O.
- Allow the sample to stand overnight.
- Transfer the Calgon® -treated sample to a metal dispersing cup.
- Mix for 5 min with an electric mixer (milkshake machine).
- Transfer the suspension to a cylinder and bring the volume to 1 liter w/dH₂O.
- Allow the suspension to equilibrate to room temp (20-25 C).
- Place a clean hand (or large stopper) tightly over the top of the cylinder and turn over.
• Continue with vigorous end-for-end turnovers for several minutes (mix well).
• Finish with 2-3 smooth gentle turnovers.
• Record the exact clock time when stirring is complete.
• Add a drop or two of amyl alcohol to the cylinder if foaming is present on the top surface.
• Lower the hydrometer gently into the suspension.
• Take a hydrometer reading at 40 sec ($R_{40S}$).
• Remove the hydrometer after the reading, rinse it, wipe dry w/a kim-wipe.
• Reinsert the hydrometer gently at 7 h and take another reading ($R_{7H}$).
• Calculate the percent sand, silt, and clay:
  \[
  \text{Sand} (\%) = 100 - \left(\frac{(R_{40S} - R_L)}{(100/\text{oven-dried soil wt. in g})}\right)
  \]
  \[
  \text{Clay} (\%) = \frac{(R_{7H} - R_L)}{(100/\text{oven-dried soil wt. in g})}
  \]
  \[
  \text{Silt} (\%) = 100 - (\text{sand} \% + \text{clay} \%)
  \]

Supplies needed:

• Calgon® (sodium-hexametaphosphate) (Fisher #04-322B)
• Amyl alcohol (50 ml) (usually avail. in soils lab)

Materials needed:

• Standard soil hydrometer (ASTM No. 1 152H)
• Electric stirrer (milkshake machine)
• 1 liter cylinders

**Mehlich III Extractable Elements (P, Ca, Mg, K, Al)**

The Mehlich III method was developed by Melich in 1984 as a multi-element soil extraction procedure. This method provides nearly identical results (for K, Ca, Mg) relative to an ammonium acetate extraction and provides a relatively simple and accurate determination of P (which ammonium acetate does not; thus, a separate extraction is not required).
Begin by producing a volume of Mehlich III extracting solution.

- Dissolve 55.56 g of ammonium flouride (NH$_4$F) in 600 ml of dH$_2$O.
- Add 29.23 g of EDTA to mixture, dissolve, bring to 1 liter w/ dH$_2$O.
- Store in a nalgene bottle, Mark as "M-3 Stock."
- Add 8 liters dH$_2$O to a plastic carboy.
- To the carboy, add:
  - 200.1 g ammonium nitrate (NH$_4$NO$_3$)
  - 100 ml of M-3 Stock
  - 115 ml acetic acid (CH$_3$COOH)
  - 82 ml of 10% nitric acid (10 ml concentrate 70% HNO$_3$ in 100 ml dH$_2$O)
- Adjust up carboy to 10 liters with dH$_2$O, mix thoroughly.

The next step is to do the extraction procedure.

- Weigh 5 g air-dried & sieved soil into a 125 ml Erlenmeyer flask.
- Add 50 ml of Mehlich III extracting solution (NOTE: for future calculations, this yields a 1:10 dilution!).
- Shake immediately on an oscillating flatbed shaker at 120 OPM for 5 min.
- Gravity filter the suspension through Whatman #542 filter paper directly in to 25 x 150 mm borosilicate glass culture tubes.
- Use a long-stem funnel in the flask, pleat the filter paper for good feed rate.
- This step may require up to 1 hr for complete filtration.
- Cap with a #4 rubber stopper and label tube as to soil sample ID.
- Analyze extraction as soon as possible (<90 d).

Supplies needed:

- Ammonium nitrate (NH$_4$NO$_3$)
- Ammonium flouride (NH$_4$F)
- Acetic acid (CH$_3$COOH)
Materials needed:

- EDTA
- Nitric acid (HNO₃, conc)
- Whatman #542 (125 mm) filter paper (Fisher #09-852H)
- Borosilicate glass culture tubes, 25 x 150 mm (Fisher #14-961-34)

Phosphorous Determination (colorimetric method)

Phosphorous analysis requires additional solutions to be made:

First, mix up solution "A":

- Dissolve 12 g of ammonium molybdate (NH₄)₆ Mo₇O₂₄ · 4H₂O in 250 ml dH₂O.
- In a 100 ml flask, dissolve 0.2908 g of potassium antimony tartrate in 80 ml of dH₂O.
- Obtain a 2 liter vol. flask, add 1000 ml of 2.5 M H₂SO₄ (141 ml conc H₂SO₄ /L).
- Add the above two solutions to the volumetric flask.
- Bring V-flask to 2 L with dH₂O; store in dark at 4 C.

Second, mix up solution "B":

- Dissolve 1.056 g ascorbic acid in 200 ml of solution "A" (prepare solution "B" daily as needed).

Third, prepare a dilution series of P:

- Use certified P standard.
- Prepare standard solutions of 0, 2, 4, 6, 8, and 10 mg g⁻¹
Conduct phosphorous assay:

- Before beginning colorimetric assay, prepare the spectrophotometer (Spec-20D).
- Remove the visible wavelength bulb and install the far wavelength bulb & filter.
- Warm up the machine for 15-20 min.
- Set the Spec-20D to 882 nm.
- Standardize to 100%T (0% A) using Mehlich III extractant in a spec-tube.
- Begin the colorimetric assay...
- Pipet 2 ml of extracted filtrate from culture tubes (see above) into a 25 ml V-flask.
- Add 15 ml dH$_2$O and 4 ml solution "B".
- Bring volume up to 25 ml w/dH$_2$O.
- Allow 10 min for color development. (CAUTION: at this stage, the colorimetric reaction has begun; can not stand > 20 min!)
- Read absorbance at 882 nm on Spectronic 20D.
- Determine P concentration via P standard curve.

Supplies needed:

- Ammonium molybdate ((NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O)
- Potassium antimony tartrate
- H$_2$SO$_4$ concentrated
- Ascorbic acid
- Phosphorous standard (1000 ppm)

Materials needed:

- Milton-Roy Spectronic 20D spectrophotometer
- Long wavelength bulb & filter for Spec-20
- 2 L volmetric flask
- 2 L Erlenmeyer flask w/stopper
- Pipetter (2 ml) w/tips
- Spectrophotometer tubes & rack
- Lab balance (0.01 g)
Ca, Mg, K, and Al Determination (atomic absorption and emission)

By its very nature, the atomic absorption spectrophotometer (AA) is a bit complicated and can be dangerous if improperly used. You should seek the guidance of someone trained in its use prior to beginning any lab procedure! While the basic theory is the same, the specifics of use vary considerably from instrument to instrument. The directions provided below pertain specifically to the Varian SpectrAA Model 20 located in Porter Hall, Room 412. Prior to beginning, it is assumed that you have already completed the Mehlich III extraction procedure (see above) and thus, all of your samples exist as undiluted extracts. Prior to beginning the analysis, you will probably need to create a dilution series for each extract such that there are 1:10, 1:100, 1:1000 dilutions in addition to the undiluted extract. This needs to be done because you will need to make sure that you are within range for your standard curve for any individual sample.

Dilutions:

- Line up your extract samples in the first row of test tube racks.
- Fill up the racks with borosilicate glass culture tubes (25 x 150 mm).
- There should now be extracts in front row, and 3 rows of tubes behind each sample.
- Create your dilution series using distilled water (1:10, 1:100, 1:1000).
- (I have not found the use Mehlich III for the dilution series to changes the results.)
- Make sure there is at least 25 ml of sample for each dilution.
- Place a black #4 rubber stopper in each. Store at 5C until ready for analysis.

Procedures for absorption (Ca, Mg, Al):

- Clean, check, and service the AA as needed.
• Make sure there is sufficient acetylene and air, order replacements if needed.
• Power up the AA, allow 15 minutes to warm up.
• Install the Ca/Mg lamp in the turret; swing wheel until it is aligned with cushion
  (Be careful and gentle! The bulbs are expensive. Avoid touching the glass.)
• Hit "index" on the keypad.
• Select "program directory."
• Select either program 20 (if doing Ca) or program 21 (if doing Mg).
• Select "recall program."
• Select "instrument parameters."
• Select "notes."
• Clean the burner groove using the brass card.
• Use the "VERT" adjustment knob to adjust beam ca. 1 cm above burner groove. (Use a white 3 x 5 card to view beam location.)
• Under the hood, adjust the wavelength value to that shown on the screen. (Move the metal pin to lock and unlock the wavelength adjustment know.)
• Adjust the slit width to the value indicated on the screen. (Move pin behind wavelength controller.)
• Turn on air tank (first!) and adjust pressure to 28 PSI.
• Turn on acetylene tank to full open (to start).
• Ignite burner by depressing the "ignite" button (if this fails, use a sparker).
• Once ignited, turn down the acetylene flow to about 1.5.
• Select "optimization" to reduce photomultiplier voltage as low as possible.
• To do this, aspirate with ddH₂O, adjust wavelength to maximize bar on screen.
• Each time bar is maxed, select "rescale."
• Repeat process as often as needed (at least several times).  
• Then adjust lamp knobs to maximize the onscreen bar.
• To do this, adjust the black knobs on the side of the lamp turret wheel.
• Maximize bar, rescale, repeat as necessary.
• Now select "standards."
• Select "calibration" then read blanks (dH₂O) and standards.
• All standard deviations should fall below 10.
• If the standard curve looks good, select "solution type" to return.
• If you are having problems, redo above adjustments, check standards, redo.
• If the standard curve graph is acceptable, select "analytical results" & proceed.
• Aspirate each sample, hit the "read" button, if out of range, select a lower dilution.
• When finished with a session, turn off acetylene at AA then air at AA.
• Turn off acetylene at tank then air at tank. Power off the AA-20 unit.

Procedures for emission (K):

The procedures for emission are virtually identical to the above. *The only substantive difference is that there is no lamp used.*

Power up AA as above. Recall program 22 for K analysis. Perform adjustment steps as above. Perform optimization steps as above. Perform standards and calibration steps as above. Read samples as above. Power off as above. Tips: Readings for blanks should be as close to 0.000 as possible. If you are having blank problems, try new blanks in clean glassware. Negative readings are not acceptable. If you get lost at a step, hit the "index" key and go to the appropriate page.

Supplies & Materials:

• Same as for above methods.

**References**

*Collecting, handling, and preparation of soils*

Nitrate Nitrogen (cadmium reduction method)


Soil moisture (gravimetric method)


Soil pH (glass electrode method)


Organic Matter and Ash Content (dry-ash method)


Soil Texture (hydrometer method)


Ca, Mg, K, and Al Determination (atomic absorption and emission)

Mulgrave, Victoria, Australia.