



# Laboratory Procedures



## Virginia Tech Soil Testing Laboratory

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## Introduction

The procedures for soil analysis used in the Soil Testing Laboratory were established in the early 1950s\*. Although the chemical principles have not changed, procedures have been revised over the years to utilize advances in instrumentation which allow more accurate and rapid chemical determinations.

A routine test, consisting of eleven separate analyses, is performed on all samples. In addition, two separate tests are offered on a request basis. These tests are applicable only under certain conditions for which research and calibration work has been conducted. The routine and special tests consist of the following:

### Routine Test

- water pH (WpH)
- buffer pH (BpH)
- phosphorus (P)
- potassium (K)
- calcium (Ca)
- magnesium (Mg)
- zinc (Zn)
- manganese (Mn)
- copper (Cu)
- iron (Fe)
- boron (B)

### Special Tests

- soluble salts
- organic matter

\*Rich, C.I., 1955. Rapid soil testing procedures used at Virginia Polytechnic Institute. Virginia Agriculture Experiment Station. Bull. 475, p. 8.

## Sample Preparation

Soil samples arrive in 1/2-pint cardboard cartons. Generally, Soil Sample Information Sheets (SSIS) are packaged with the samples. The cartons are opened in a separate preparation area and placed in drying trays. Twenty-eight unknown samples plus two control samples are placed in each drying tray. The two control samples are one known internal reference sample and either a blank or replicate sample. At this time, each sample is assigned a laboratory number which, along with the year, is stamped on the SSIS. The samples are numbered consecutively each calendar year, beginning with 1 on January 1.

The trays of samples are placed in a cross-flow forced-air drying cabinet through which room-temperature filtered air is drawn. The air can be heated 5° to 8°C above the ambient temperature for drying extremely wet samples. Samples remain in the drying cabinet overnight or until air dry.

Air-dried (at 20° to 40°C) samples are crushed with a hammermill-type crushing machine and passed through a 10-mesh (2-mm opening) stainless steel sieve. The samples are then returned to the original sample boxes until the various subsamples are measured out.

## Water pH (WpH) Determination

**Buffer Solutions:** Color-coded buffer solutions of pH 4.0, 7.0, and 10.0 are purchased from commercial sources.

**Electrode Internal Filling Solution:** Use Thermo Orion's 3M KCl, (with no silver), Ross™ Sure-Flow® Internal Filling Solution, Cat. No. 810007.

**Electrode Soaking Solution:** Use 5 g of KCl in a liter of pH 7 buffer solution.

### Procedure:

Daily, do a two-point calibration of the pH meter using fresh buffer solutions of pH 4 and 7, and recheck the calibration before starting every batch of samples.

Scoop 10 cm<sup>3</sup> of soil from the prepared sample into a 50-ml beaker. With an automatic pipetting machine add 10 ml of distilled water for a 1:1 (vol/vol) ratio. Thoroughly mix the solution with a glass/plastic rod or mechanical stirrer and allow it to sit for a minimum of 10 minutes and a maximum of 2 hours.

The automated pH analyzer is set to stir solutions for a 5-second equilibration delay before starting to take pH readings. It then continues to stir the soil suspension while the software waits for 10 readings to be stable within 0.02 pH units. Probes are automatically washed after a pH reading greater than 8.0 or less than 4.0. Readings are electronically recorded to the 0.01 pH unit. The pH readings of quality-control samples are manually checked before uploading the sample data to verify that they are within  $\pm 0.1$  pH unit of the expected value. This includes reading a pH 10 buffer solution if a sample had a pH reading above 7.00. After use, place the electrodes in the soaking solution.

### Notes:

- For fine-textured soils containing a high level of organic matter, it may be necessary to add an additional 10 ml of distilled water to make a suspension.
- The TPS pH meter has a temperature sensor for automatic temperature compensation (ATC). This ATC probe should sit in a flask of ambient temperature water within the LabFit pH Analyser next to the soil samples being measured.
- If a pH probe's reading becomes sluggish, unstable, or not reproducible (possibly indicating that the liquid reference junction has become clogged), depress the electrode's top cap to flush the junction.

## Buffer pH (BpH) Determination

### Mehlich Buffer Preparation:

Using a 4-liter volumetric flask, add:

~ 2 liters of distilled water (DW);

10 ml of glacial acetic acid,  $\text{CH}_3\text{COOH}$ , 99.5%, 17.4N;

39 ml of 50% triethanolamine (1 TEA : 1 DW);

72.0 g of sodium glycerophosphate, hydrate,  $\text{C}_3\text{H}_5(\text{OH})_2\text{PO}_4\text{Na}_2 \cdot x\text{H}_2\text{O}$ , FW=216.04(anhy.); or 1,2,3-Propanetriol mono (dihydrogen phosphate) disodium salt,  $(\text{HOCH}_2)_2\text{CHOPO}_3\text{Na}_2$ ; or Glycerol phosphate Disodium salt Hydrate,  $\text{C}_3\text{H}_7\text{O}_6\text{PNa}_2$ , CAS #: 154804-51-0 {Sigma's 1 kg, G6501 or Gallard-Schlesinger's 50 kg GSODGLYERO via Doe & Ingalls};

172.0 g of ammonium chloride ( $\text{NH}_4\text{Cl}$ );

48.0 g of calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ); {or alternatively use 80.0 g  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  }.

Stir using a stir-bar and stir-plate until all salts are dissolved and allow the solution to warm up to room temperature.

Bring to the 4-liter volume with distilled water.

Adjust to pH  $6.60 \pm 0.04$  when diluted 1:1 with distilled water. Use drops of acetic acid to lower the pH or drops of 1:1 aqueous TEA to raise the pH.

Use an acid standard to check the preparation of the buffer mixture as follows: combine 10 ml of buffer, 10 ml of distilled water, and 10 ml of commercially prepared 0.05N HCl solution. This mixture should drop the initial buffer pH by  $1.35 \pm 0.1$  units. If the pH is not within these limits, check the preparation of the buffer reagent to make certain that all ingredients were added properly.

Make only what will be needed for a week to prevent microbial growth in storage. When calcium chloride is used instead of barium chloride, containers and dispensers may need to be disinfected with dilute (10%) chlorine bleach (sodium hypochlorite) between batches of solution. Rinse very well with distilled water.

### Procedure:

On samples with a  $\text{WpH} \leq 6.94$ , add  $10 \pm 0.2$  ml of the Mehlich buffer solution using the 1:1 (vol/vol) soil-water mix from the water pH determination. Thoroughly mix the solution with a glass/plastic rod and allow it to sit for a minimum of 30 minutes. Stir the solution again immediately before reading and while the pH probe is equilibrating in the soil suspension. Record the first stable pH reading to the nearest 0.01 unit. Verify calibration of pH electrodes before measuring buffer pH's. Check the pH of the buffer solution on the daily blank sample. A rise in its pH indicates fungal growth in the buffer.

# Determination of P, K, Ca, Mg, Zn, Mn, Cu, Fe, B, and Al

## Extracting Solution (Mehlich 1, 0.05N HCl in 0.025N H<sub>2</sub>SO<sub>4</sub>):

Measure approximately 15 liters of distilled water into a 20-liter plastic container. Add 14.0 ml of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), 82.0 ml of concentrated hydrochloric acid (HCl), and distilled water to make a 20-liter volume and mix thoroughly.

## Extraction Procedure:

Measure one 4-cm<sup>3</sup> scoop of soil into a 60-ml straight-walled plastic extracting beaker, and add 20 ml of the Mehlich 1 extracting solution with an automatic pipetting machine. The samples are shaken on a reciprocating shaker with a stroke length of 3.8 cm for 5 minutes at 180 oscillations per minute and filtered through Whatman No. 2 (or equivalent), 11-cm filter paper soon after the shaking stops.

## Analysis Procedure:

All elements are analyzed in the same extract by an ICP (inductively coupled plasma atomic emission spectrometer). Transfer filtrate from the extraction beaker to an ICP autosampler cup by using a disposable polyethylene pipette. The transfer is a two-step procedure with the first aliquot being a rinse and the second aliquot for the actual transfer. Pipette 4 ml of filtrate and discard into a waste beaker. Pipette another 4 ml of the same filtrate into the autosampler rack's polystyrene sample cups.

Once all sample filtrates have been transferred, cover the autosampler rack with plastic wrap to prevent air-borne contaminants (dust, lint, etc.) from getting into the solutions. This is important to prevent ICP nebulizer clogging and contamination.

Samples may be stored overnight by covering them with plastic wrap, parafilm, or capping and placing them in a refrigerator. After refrigeration, allow the samples to equilibrate to room temperature before ICP analysis.

## Elemental Analysis by ICP:

The ICAP 61E (a simultaneous spectrometer), equipped with a Thermo Elemental autosampler, is set up to analyze approximately 30 samples for 10 elements about every 25 minutes (an 11-second rinse [during exposure time] followed by a 25-second flush time and one 5-second sample exposure with one 5-second background exposure). Read and verify a quality control solution after every tray of 30 samples.

## ICP Working Standards:

The ICP is calibrated with the following series of standards (Note: atomic absorption standards are not sufficiently pure for ICP standards; use only spectrally pure, plasma-quality standards).

Soil #1: Final solution concentration: 0.05 N HCl and 0.025 N H<sub>2</sub>SO<sub>4</sub>.

Use the Mehlich 1 (M1) extracting solution or to approximately 250 ml of deionized water in a half-liter volumetric flask, add 2 ml of concentrated reagent grade HCl, and 0.35 ml of concentrated reagent grade H<sub>2</sub>SO<sub>4</sub>, dilute to volume with deionized water and mix well.

Soil #2: Final elemental concentration in solution: 30 µg ml<sup>-1</sup> P, 2 µg ml<sup>-1</sup> Zn, 2 µg ml<sup>-1</sup> B.

To approximately 250 ml of M1 extracting solution in a half-liter volumetric flask, add 15 ml of 1000 µg ml<sup>-1</sup> P calibration standard, 1 ml of 1000 µg ml<sup>-1</sup> Zn calibration standard, 1 ml of 1000 µg ml<sup>-1</sup> B calibration standard and dilute to volume with extracting solution and mix.

Soil #3: Final elemental concentration in solution: 300  $\mu\text{g ml}^{-1}$  Ca, 100  $\mu\text{g ml}^{-1}$  K, 50  $\mu\text{g ml}^{-1}$  Mg, 10  $\mu\text{g ml}^{-1}$  Al, 10  $\mu\text{g ml}^{-1}$  Mn.

Add to a half-liter volumetric flask with approximately 250 ml of M1 extracting solution 15 ml of 10,000  $\mu\text{g ml}^{-1}$  Ca calibration standard, 5 ml of 10,000  $\mu\text{g ml}^{-1}$  K calibration standard, 2.5 ml of 10,000  $\mu\text{g ml}^{-1}$  Mg calibration standard, 5 ml of 1,000  $\mu\text{g ml}^{-1}$  Al calibration standard, and 5 ml of 1000  $\mu\text{g ml}^{-1}$  Mn calibration standard; dilute to volume with extracting solution and mix.

Soil #4: Final elemental concentration in solution: 10  $\mu\text{g ml}^{-1}$  Cu, 25  $\mu\text{g ml}^{-1}$  Fe.

Add to a half-liter volumetric flask with approximately 250 ml of M1 extracting solution 5 ml of 1000  $\mu\text{g ml}^{-1}$  Cu calibration standard and 12.5 ml of 1000  $\mu\text{g ml}^{-1}$  Fe calibration standard; dilute to volume with extracting solution and mix.

### ICP Quality Control Standard:

The quality control solution is prepared with spectrally pure, ICP-quality, calibration stock solutions. (Note: For the elements P, K, Ca, and Mg, use standard stock solutions from a manufacturing source other than the one used to prepare the working standards.) Add to a half-liter volumetric flask with approximately 250 ml of Mehlich 1 extracting solution the following amounts of each stock solution then dilute to volume with extracting solution and mix well:

Element	Final Concentration ( $\mu\text{g ml}^{-1}$ )	High Purity Reference Solution
P	10	5 ml of 1,000 $\mu\text{g ml}^{-1}$
K	25	1.25 ml of 10,000 $\mu\text{g ml}^{-1}$
Ca	200	10 ml of 10,000 $\mu\text{g ml}^{-1}$
Mg	20	1 ml of 10,000 $\mu\text{g ml}^{-1}$
Zn	1	0.5 ml of 1,000 $\mu\text{g ml}^{-1}$
Mn	1	0.5 ml of 1,000 $\mu\text{g ml}^{-1}$
Cu	1	0.5 ml of 1,000 $\mu\text{g ml}^{-1}$
Fe	5	2.5 ml of 1,000 $\mu\text{g ml}^{-1}$
B	1	0.5 ml of 1,000 $\mu\text{g ml}^{-1}$

### Calculation of Elemental Concentrations:

For each element, the calculation for ppm in soil is as follows:

ppm in solution  $\times 5 =$  ppm in soil on a volume basis ( $\text{mg}/\text{dm}^3$ )

ppm in solution  $\times 4 =$  ppm in soil on a weight basis ( $\text{mg}/\text{kg}$ )

where 4 is the dilution factor assuming a soil scoop density of 1.25  $\text{g}/\text{cm}^3$ .

To convert from ppm (wt. basis) to lbs/acre the equation is:

ppm in soil  $\times 2 =$  lbs/acre

where weight of an acre furrow slice (6 2/3-inch depth) is assumed to be 2 million pounds.

## Estimation of Effective CEC by Summation

### Theory:

The Cation Exchange Capacity (CEC) can be reasonably estimated by summation of the Mehlich 1 extractable bases, or non-acid generating cations (Ca, Mg and K), plus the exchangeable acidity estimated from the Mehlich buffer pH after conversion of all analytical results to meq/100 cm<sup>3</sup> or cmol(+)/kg.

This calculated method is closer to a measured Effective CEC, which is measured at the present pH of the soil, than it is to the soil's potential CEC, which is measured in solutions buffered at pH 7.0 or higher.

This method is inappropriate for soils with a high soluble salts level or for alkaline soils because these soils may be over-fertilized, calcareous, gypsiferous, or relatively unweathered and could result in an erroneously high CEC value by the release of nonexchangeable cations.

### Calculation:

Estimated Soil E-CEC = Acidity + Ca + Mg + K (in the units of meq/100 g soil or cmol/kg)

Acidity (meq/100 g of soil) =  $37.94 - (5.928 \times \text{BpH})$

where BpH = Mehlich buffer pH reading for an individual soil sample.

meq Ca/100 g = lb Ca per Acre  $\div$  401

meq Mg/100 g = lb Mg per Acre  $\div$  243

meq K/100 g = lb K per Acre  $\div$  782

## Soluble Salts

### Conductivity Standard:

Use a commercially prepared NIST traceable conductivity standard of 1,000 or 1,420  $\mu\text{siemens/cm}$ .

or

Prepare potassium chloride standard solution (0.01 N KCl): Dissolve 0.7456 g of potassium chloride (KCl) in deionized water in a 1-liter volumetric flask. Mix well and dilute to volume. The conductivity of this solution at 25°C is 1,412  $\mu\text{siemens/cm}$ .

### Procedure:

Measure one 20-cm<sup>3</sup> scoop of soil into a 50-ml beaker, add 40 ml of distilled water for a soil:water ratio of 1:2 (vol/vol). Include at least one internal soil reference (“test”) sample per batch of unknown soil samples. Stir the solution and allow the suspension to settle for at least 1 hour. Check the conductivity meter’s calibration against the conductivity standard. At 25°C, the standard has an electrical conductivity of 1.00 or 1.41 mmho/cm (or mS/cm). Set the meter in the Temperature Compensation Conductivity mode, and cell constant (C) to 1.00/cm. The electrical conductivity (EC) of the supernatant liquid of the soil-water solution is determined with the meter set on the  $\mu\text{S/cm}$  scale. Record the EC as one tenth of the meter’s reading, (move the decimal one place to the left on the meter’s display), in order to give the results in mhos  $\times 10^{-5}$  units. The ppm soluble salts in the soil are calculated from the following equation:

$$\text{ppm soluble salts in soil} = \text{EC} \times 6.4 \times 2$$

In this equation, EC represents the conductivity reading in mhos  $\times 10^{-5}$ , 6.4 is the factor for converting the conductivity measurement to ppm soluble salts, and 2 represents the water volume dilution factor. Report as ppm soluble salts in soil.

### Useful Equations:

$$\text{EC (mho} \times 10^{-5}/\text{cm)} / 100 = \text{mmho/cm}$$

$$\text{ppm (mg salt/liter)} / 1280 = \text{mmho/cm}$$

$$0.1 \text{ S/m} = 1 \text{ dS/m} = 1 \text{ mS/cm} = 1 \text{ mmho/cm}$$

Resistance of a solution is the reciprocal of the electrical conductivity; therefore,

$$0.1 \mu\text{mho} = 10.0 \text{ Mohm.}$$

## Soil Organic Matter (SOM) by Walkley-Black (WB)

Reagent A: Sodium dichromate solution (0.67M): Dissolve 500 g of reagent grade sodium dichromate ( $\text{Na}_2\text{Cr}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in tap water to a volume of 2 1/2 liters.

Reagent B: Concentrated reagent grade sulfuric acid ( $\text{H}_2\text{SO}_4$ ).

### Procedure:

The procedure is a modified Walkley-Black method. Measure one 1.5-cm<sup>3</sup> scoop of soil into a 200-ml test tube. Under a hood, add 20 ml of Reagent A to the soil followed by 20 ml of Reagent B. Allow the solution to cool at least 40 minutes. After cooling, add 100 ml of tap water, mix the solution, and allow to stand overnight (or at least 8 hours). After incubation, withdraw an aliquot of the supernatant using a syringe-type pipette and transfer it to a colorimeter vial. Take readings using a colorimeter set to a 645 nm wavelength. The percentage of organic matter is determined by reference to Table 2.

Table 2. Colorimeter readings and percent organic matter.

Colorimeter Reading	Organic Matter, %	Colorimeter Reading	Organic Matter, %	Colorimeter Reading	Organic Matter, %
100	0.0	57	2.8	29	6.7
99-95	0.1	56	2.9	28	6.9
94-91	0.2	55	3.0	27	7.1
90-89	0.3	54	3.1	26	7.3
88-87	0.4	53	3.2	25	7.5
86	0.5	52	3.3	24	7.7
85-84	0.6	51	3.4	23	7.9
83	0.7	50	3.5	22	8.1
82-81	0.8	49	3.6	21	8.4
80	0.9	48	3.7	20	8.8
79-78	1.0	47	3.8	19	9.1
77	1.1	46	3.9	18	9.5
76-75	1.2	45	4.0	17	9.8
74	1.3	44	4.1	16	10.2
73-72	1.4	43	4.2	15	10.5
71	1.5	42	4.3	14	10.9
70-69	1.6	41	4.4	13	11.2
68	1.7	40	4.5	12	11.6
67	1.8	39	4.7	11	11.9
66	1.9	38	4.9	10	12.3
65	2.0	37	5.1	9	12.6
64	2.1	36	5.3	8	13.0
63	2.2	35	5.5	7	13.3
62	2.3	34	5.7	6	14.1
61	2.4	33	5.9	5	15.0
60	2.5	32	6.1	4	15.0
59	2.6	31	6.3	3	15.0
58	2.7	30	6.5	2-1	15.0

## Soil Organic Matter (SOM) by Weight Loss On Ignition (LOI)

### Procedure:

Tare balance and weigh 50-mL beakers. Scoop 5 cm<sup>3</sup> of air-dried, 2-mm sieved soil into a beaker. Dry for a minimum of two hours at 150°C ±5°C. Maintain at 100°C until weighing. Record the weight of the beaker plus the warm soil sample to ±1 mg. Heat at 360°C for two hours after the temperature reaches 360°C ±5°C. Cool to 105°C and maintain at 105°C until weighing. Weigh the beaker and warm ash in a draft-free environment to ±1 mg. Calculate and report %LOI as percent organic matter to the nearest tenth of a percent.

### Calculations:

Dried Soil (Soil<sub>d</sub>) = (Wt of Beaker + Wt of Soil at 150°C) - Wt of Beaker

Ashed Soil (Soil<sub>a</sub>) = (Wt of Beaker + Wt of Soil at 360°C) - Wt of Beaker

Percent weight loss on ignition (%LOI):

$$\text{LOI (\%)} = \frac{\text{Soil}_d - \text{Soil}_a}{\text{Soil}_d} \times 100$$

### Note:

The LOI (a gravimetric, dry oxidation) method is used to estimate the soil organic matter content for all samples except for those coming from commercial farmland in the Piedmont counties of Virginia. The Walkley-Black (a wet, chemical oxidation) method is used in those cases, due to the presence of gibbsite (Al<sub>2</sub>O<sub>3</sub> • 3H<sub>2</sub>O) in the clay fraction of soil material in that area of the state. Gibbsite has been reported to lose substantial amounts of water at around 300°C.

## Instruments for Soil Analyses

### Analysis

### Instrument

Soil Drying

Cross-flow forced-air soil drying cabinet, developed at Virginia Tech

Soil Grinding

Custom Lab Equipment DC-1 HD Dynacrush

pH Auto-analyzer

LabFit Pty Ltd, model AS-3000 Automated Dual pH Analyser

pH Meter

TPS Pty Ltd, model WP-80D, Dual pH-mV and temp. meter

pH Electrode

Thermo Orion model 8165BNWP, Ross™ combination pH electrode, Sure-Flow®, with epoxy-body and BNC connector

Nutrient Extraction

Eberbach Reciprocating, Variable Speed Shaker No. 6000

Elemental Analysis of P, K, Ca, Mg, Zn, Mn, Cu, Fe, B & Al

Thermo Elemental ICAP 61E (Inductively Coupled Argon Plasma Atomic Emission Simultaneous Spectrometer) using Thermo's ICP Manager 61 software, and equipped with a TJA-300 autosampler.

Soluble Salts

YSI 3100 Conductivity Instrument with a YSI 3254 Pyrex 5-ml Fill Cell

Organic Matter - WB

Spectronic® 20 Genesys™ Colorimeter

Organic Matter - LOI

Blue M Electric High Temperature (up to 704°C), Ultra-Temp, forced-air drying oven, model CW-6680F, with Pro 550 microprocessor-based controller.

Organic Matter – LOI

PG503-SDR Mettler Toledo (MT) analytical balance controlled by MT's BalanceLink software (v2.20).

## ICP Parameters

The ICP is housed in an instrument room maintained at 21°C (70°F) ± 1°C (2°F). Swings in both temperature and humidity can affect the analytical results. To enhance precision the solutions are introduced to a cross flow nebulizer with a peristaltic pump. The ICP is profiled using a Hg wavelength.

The following analytical lines are used:

Element	Wavelength (nm)	Physical Channel Number	Analytical Range (µg ml <sup>-1</sup> )
P	214.914	38	0.06 - 1000
K	766.490	24	0.3 - 1000
Ca	373.690	43	0.1 - 1500
Mg	279.079	14	0.01 - 350
Zn	213.856	33	0.004 - 150
Mn	257.610	37	0.001 - 150
Cu	324.754	4	0.002 - 150
Fe	259.940	12	0.005 - 150
B	249.678	11	0.006 - 150
S	182.04	7	0.1 - 500
Na	588.995	16	0.01 - 200
Li	670.784	20	0.006 - 150
Al	396.153	41	0.025 - 500
Al	308.215	17	1.0 - 5000
Hg	546.074	13	Monitor

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### **CEC by Summation:**

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