

Soil Test Methods

From the Southeastern United States



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Chapter 4.3

Mehlich-3

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

Application and Principle

The Mehlich-3 method estimates availability of most plant nutrients in soil using a solution with salt, dilute acid, fluoride, and EDTA buffered with acetic acid at pH 2.5. The method was developed by Mehlich in North Carolina to improve the Mehlich-1 extractant (0.05 M HCl and 0.0125 M H₂SO₄) that did not show a good correlation between extracted phosphorus and crop growth for all the variable soil types across the state (Mehlich, 1984). Lower critical soil test levels for Mehlich-1 in fine-textured soil compared to heavy-textured soil (Kamprath and Watson, 1980) indicated Mehlich-1 was not dissolving a portion of bioavailable P on fine-textured soil that was associated with aluminum. Fluoride was added in the Mehlich-3 extractant to complex aluminum in solution which acted to dissolve aluminum phosphates and prevent refixation of dissolved P (Nelson et al., 1953). Fluoride can precipitate as fluorite (CaF₂) on the surface of calcium carbonate in soil and the resultant precipitate has a high affinity for resorbing dissolved P in the extract at higher pH (Gunawan et al., 2010; Smillie and Syers, 1972; Turner et al., 2005; Turner et al., 2010). To prevent P resorption, acetic acid was added to the Mehlich-3 extractant to serve as a pH buffer to keep pH at 2.5 (Mehlich, 1978a; Mehlich, 1978b). Ammonium nitrate salt in Mehlich-3 serves to extract exchangeable cation nutrients such as calcium (Ca), magnesium (Mg), and potassium (K). The chelate, EDTA, was added to improve the extraction of micronutrients from soil with a particular emphasis on copper, manganese, and zinc (Mehlich, 1984). There was a Mehlich-2 extractant that was developed in between Mehlich-1 and Mehlich-3 that is not currently used. The Mehlich-2 extractant contained salt, dilute acid, and fluoride buffered with acetate at pH 2.5 (Mehlich, 1978b). The salt and acids were chlorides and EDTA was not present. The chloride and acid had undesirable corrosive properties in the lab. Changing the salts to nitrate and adding EDTA resulted in the Mehlich-3 extractant (Mehlich, 1984).

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

Equipment and Apparatus

1. Soil scoop with 2.5 cm³ capacity if measuring soil by volume
2. Analytical balance with 0.01 g resolution or 2 g calibrated scoop if measuring soil by weight

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

Reagents

1. *Mehlich-3 extractant*: Following are directions for making 20 L. Multiply or divide quantities by the appropriate factor for making larger or smaller volumes of the extracting solution. The solution contains 0.2 M acetic acid, 0.25 M ammonium nitrate, 0.015 M ammonium fluoride, 0.013 M nitric acid, and 0.001 M ethylenediaminetetraacetic acid.
Stock solution with 3.75 M NH₄F and 0.25 M EDTA
 - a. In a 2 L volumetric flask, add about 1200 mL H₂O
 - b. Add 277.8 g ammonium fluoride (NH₄F) and thoroughly stir to dissolve.
 - c. Add 146.1 g of ethylenediaminetetraacetic acid (EDTA, (HO₂CCH₂)₂NCH₂CH₂N(CH₂CO₂H)₂) and thoroughly stir to dissolve.
 - d. Bring solution to 2 L volume with deionized water and thoroughly stir.
 - e. Store stock solution in plastic bottle.Mehlich-3
 - f. Fill a 20-L calibrated plastic carboy with approximately 12 L of deionized water.
 - g. Add 400 g of ammonium nitrate (NH₄NO₃) and thoroughly stir to dissolve.
 - h. Add 80 mL of stock solution containing with 3.75 M NH₄F and 0.25 M EDTA. Thoroughly stir to dissolve.
 - i. Add 230 mL concentrated glacial acetic acid (CH₃COOH) and 16.4 mL concentrated HNO₃. Thoroughly stir.
 - j. Bring solution to 20-L with deionized water and thoroughly stir.
 - k. The pH of the extracting solution is approximately 2.5.
2. *Reagents for molybdate-blue spectrophotometric analysis of P*: The reagents described here were taken from Tucker (1992). The reagents were developed from Watanabe and Olsen (1965) who modified the original method by Murphy and Riley (1962) with a reagent made on a daily basis. Reagent A has more concentrated components than the comparable reagent from Watanabe and Olsen (1965) since the reagent is diluted when creating Reagent C.
Reagent A (sulfuric-molybdate-tartrate solution)
 - a. In a 2 L volumetric flask, dissolve 100 g of ammonium molybdate ((NH₄)₆Mo₇O₂₄ · 4H₂O) in 500 mL of deionized water.
 - b. Dissolve 2.425 g of antimony potassium tartrate (K(SbO)·C₄H₄O₆·½ H₂O) in the molybdate solution just prepared.

- c. Slowly add 1400 mL of concentrated H₂SO₄ (18 M). Stir to ensure thorough mixing. The solution will heat up upon adding H₂SO₄.
- d. Let the solution cool to room temperature then dilute to 2 L with deionized water. Store in an opaque polyethylene or Pyrex glass bottle in a dark and refrigerated environment.

Reagent B (ascorbic acid solution)

- a. In a 2 L volumetric flask, dissolve 176 g ascorbic acid (C₆H₈O₆) and dilute to 2 L. Store in an opaque polyethylene or Pyrex glass bottle in a dark and refrigerated environment.

Reagent C (working solution)

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

3. *Calibrations Standards for analysis via ICP-AE spectrometry (ICP-AES)*

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

Table 1. Suggested calibration standard concentrations (mg L⁻¹) for quantitative analysis of Mehlich-3 soil extracts using ICP-AES.

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

Procedure

Extraction

1. Scoop 2.5 cm³ or measure 2 g of processed soil (dried, < 2 mm) and add to an extraction flask.
2. Add 25 mL of Mehlich-3 extracting solution to the flask.
3. Twenty-five mL of Mehlich-3 and 2.5 cm³ of soil were used in the originally developed method (Mehlich, 1984). These quantities result in a 10:1 extractant/soil volume ratio. As the Mehlich-3 extract became adopted by other laboratories, a ratio of 10:1 was used with soil measured on a weight basis. For example, 20 mL of Mehlich-3 and 2 g of soil are

commonly used quantities. The weight of soil is either measured with a balance or approximated with a 1.7 cm³ scoop volume which assumes density of processed soil is 1.18 g cm⁻³ (Peck, 1998). Whether using a 10:1 ratio based on soil volume or weight, fertilizer recommendations from the test results should be based on calibrations that used soil test results with the same ratio.

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

Phosphate Analysis via Molybdate-Blue Spectrophotometry

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

Analysis via ICP-AES

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

Calculations

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

a. Volume basis

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

Volume basis, mg dm⁻³ =

$$\text{mg L}^{-1} \times (0.025 \text{ L Mehlich-3} \div 2.5 \text{ cm}^3 \text{ soil}) \times (1000 \text{ cm}^3 \div 1 \text{ dm}^3) = \text{mg L}^{-1} \times 10$$

If the density of processed soil is assumed to be 1 g cm⁻³, mg dm⁻³ is equivalent to mg kg⁻¹.

b. Weight basis

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

$$\text{Weight basis, mg kg}^{-1} = \text{mg L}^{-1} \times (0.020 \text{ L Mehlich-3} \div 2 \text{ g soil}) \times (1000 \text{ g soil} \div \text{kg soil}) = \text{mg L}^{-1} \times 10$$

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

c. Area basis

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

$$\text{Area basis, lbs acre}^{-1} = \text{mg L}^{-1} \times (2.2 \text{ lbs nutrient} \div 10^6 \text{ mg nutrient}) \times (0.020 \text{ L Mehlich-3} \div 2 \text{ g soil}) \times (1000 \text{ g soil} \div 2.2 \text{ lbs soil}) \times (2 \times 10^6 \text{ lbs soil} \div \text{acre}) = \text{mg L}^{-1} \times 20$$

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

Analytical Performance

Range and Sensitivity

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

Precision and Accuracy

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

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

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

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

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

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

Interferences

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

Interpretation

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

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

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

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

Effects of Storage

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

Safety and disposal

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

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