SCOPE

The Mehlich-3 method is a versatile method for the determination of Soil P (acidic to neutral in pH and calcareous soil, Exchangeable Cations, Trace Elements / Micronutrients and B).

PRINCIPLE

Soils need various macro and micronutrients in order to proceed with biochemical process that maintains its life; which in turn leads to greater yields and higher quality of these agricultural products.

The concentration of exchangeable bases and the cation exchange capacity (CEC) are important properties of soils and sediments. They relate information on soils ability to sustain plant growth, retain nutrients, buffer acid deposition or sequester toxic heavy metals. Cation exchange occurs due to the negative charges carried by soil particles, in particular clay minerals, sesquioxides and organic matter. The capacity of soils to bind ions is above all linked to small particles, mainly clay minerals and humus. Humus particles may be big, but they have a large inner surface where ion exchange can take place. Common base cations are calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) and sodium (Na^{+}). Most base cations, except for sodium, are nutrients important for plant growth.

Micronutrients are essential elements that are used by plants in small quantities. Micronutrients in soil are available in their cationic forms, Cu^{2+}, $Zn^{2+,}$ Fe^{2+} and Mn^{2+}. These micronutrients are essential as it serves as a co-factor in the enzymes that are responsible in biochemical processes, namely photosynthesis, nitrogen reduction, nitrogen fixation, germination and lignin formation among others.

Phosphorus is known as the master key to agriculture because lack of available P in the soils limits the growth of both cultivated and uncultivated plants. It has also been established that phosphorus is relatively unavailable for plant uptake in highly weathered soils. It is firmly fixed in soil and its proportion is relatively stable and dependent on soil reaction values. Adequate phosphorus results in higher grain production, improved crop quality, greater stalk strength, increased root growth, and earlier crop maturity.

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. This was developed by Mehlich in North Carolina to improve from the Mehlich-1 extractant (0.05 M HCl and 0.0125 M H_2SO_4) as it does not show good correlation between extracted phosphorus and crop growth for all the variable soil types across the state (Mehlich, 1984). Fluoride was added to complex aluminum in solution, thus dissolving aluminum phosphates and preventing refixation of dissolved P. (Nelson et al., 1953). However, fluoride can precipitate as Calcium Fluorite, (CaF_2) and this precipitate has a high affinity for resorbing dissolved P in the extract on higher pH. (Gunawan et al., 2010; Smillie and Syers, 1972; Turner et al., 2005; Turner et al., 2010). To prevent this, 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, Magnesium, and Potassium. The chelate, EDTA, was added to improve the extraction of micronutrients from soil with a particular emphasis on copper, manganese, and zinc (Mehlich, 1984).

The Mehlich-3 method is a versatile method for the determination of Soil P (acidic to neutral in pH and calcareous soil, Exchangeable Cations, Trace Elements / Micronutrients and B) that has been widely adopted by many laboratories

across the US and the world. The concentrations of Na, K, Ca and Mg are determined directly using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES).

EQUIPMENT

a) Analytical Balance
b) Reciprocating Mechanical Shaker, capable of 180 oscillations per minute
c) Vortex Mixer
d) Inductively Coupled Plasma – Optical Emission Spectroscopy.

LABORATORY WARE

a) Plastic scoop
b) Polyethylene carboy, 2L and 20 L capacity
c) Volumetric Bottle-Top dispenser, 25 mL
d) Extraction bottles of flasks (50-100 ml) with cap
e) Filter Funnels and vials
f) Whatman No. 1 filter paper or equivalent
g) Test tubes, 25 mL capacity
h) Test tube rack
i) Volumetric pipettes
j) Automatic pipettes, 1mL and 10 mL capacity
k) Polyethylene beaker with handle, 5L
l) Volumetric flasks, 50ml and 1L capacity
m) Aspirator
n) Parafilm

CHEMICALS AND REAGENTS

a) Ammonium Fluoride and EDTA Stock Solution, 3.75 M NH_4F and 0.25 M EDTA. In a 1 L volumetric flask, add about 600 mL water (H_2O). Add 138.9 g ammonium fluoride (NH_4F) and thoroughly stir to dissolve. Add 73.05 g of ethylenediaminetetraacetic acid (EDTA, $((HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2)$ and thoroughly stir to dissolve. Bring solution to 1 L volume with deionized water and thoroughly stir. Store stock solution in plastic bottle.
b) Mehlich-3 Extractant, 0.2 M CH_3COOH, 0.25 M NH_4NO_3, 0.015 M NH_4F, 0.013 M HNO_3, and 0.001 M EDTA. Fill a 5-L calibrated plastic beaker with approximately 3 L of deionized water. Add 100 g of ammonium nitrate (NH_4NO_3) and thoroughly stir to dissolve. Add 20 mL of stock solution containing with 3.75 M NH_4F and 0.25 M EDTA. Thoroughly stir to dissolve. Add 57.5 mL concentrated glacial acetic acid (CH_3COOH) and 4.1 mL concentrated HNO_3. Thoroughly stir. Bring solution to 5-L with deionized water and thoroughly stir. The pH of the extracting solution is approximately 2.5.
c) Calibration standards for analysis via ICP-OES.
   - 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.

- Prepare additional calibration standards by diluting the most concentrated calibration standard with Mehlich-3. See Table 1 for the recommended concentration range for calibration standards of exchangeable bases.
- Cations (K, Ca, Mg, Na) can be grouped together in the same calibration standards for ICP-AES.

Table 1. Suggested calibration standards for quantitative analysis of Mehlich-3 soil extracts using ICP-AES.

	K (mg/L)	Na (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)	P (mg/L)	B (mg/L)
Blank	0	0	0	0	0	0	0	0	0	0
Standard 1	10	10	50	10	10	2	2	2	10	2
Standard 2	25	25	125	25	25	5	5	5	25	5
Standard 3	50	50	500	100	50	10	10	10	50	10

HEALTH AND SAFETY

Wear proper personal protective equipment. Use laboratory coat, close shoes, gas mask or dust mask and appropriate gloves when performing chemical analysis to mitigate the harmful effects of exposure on chemicals.

Observe careful and proper handling of chemicals when using strong alkali bases, strong acids and oxidizing agents. Potential direct body contact (e.g. ingestion, inhalation) may cause severe irritation and inflammation to the skin, eyes, respiratory and digestive tracts. Severe exposure to these corrosive substances may cause serious health damage or death.

Avoid mixing incompatible chemicals to reduce risks of fire and explosion inside the laboratory. Sulfuric acid may react violently when mixed with water because of its high affinity for water. Always pour the acid into water to avoid splattering.

PROCEDURE

(US Southern Extension and Research Activity Information Exchange Group 6 (US SERA-IEG-6 Soil Test Methods From the Southeastern United States, 2014)

1. Weigh 2.0 g of dried and sieved (< 2 mm) soil and transfer it into an extraction bottle.
2. Add 20 mL of Mehlich-3 extracting solution to the flask/bottle
3. Add stopper to the flask/bottle and shake soil and Mehlich-3 for 5 min on a reciprocating mechanical shaker with a minimum of 180 oscillations per minute.
4. Filter the suspension using Whatman No. 1 filter paper and collect the filtrate in vials for analysis. Refilter if filtrate is cloudy.