Soil - Available P Protocol by Mehlich 3 Extraction

•updated December 2019
•adapted from Frank, Beegle & Denning 2012; https://www.naptprogram.org/files/napt/north-central-states-methods-manual-2012.pdf

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Make solutions:

Ammonium fluoride EDTA stock
• Add 13.89 g ammonium fluoride to 60 mL nanopure in a 100 mL volumetric and mix well
• Add 7.305 g EDTA to the solution
• Bring to final volume of 100 mL and mix well
• Store in dark at 4°C
• Good for ~ 500 samples

Mehlich 3 Extractant
• Dissolve 20 g ammonium nitrate into 800 mL nanopure in a 1 L volumetric
• Add 4 mL ammonium fluoride EDTA stock to the solution and mix well
• Add 11.5 mL acetic acid to the solution
• Add 0.82 mL nitric acid to the solution and mix well
• Store in dark at 4°C
• Good for ~ 40 samples

Acid Molybdate Stock
• Dissolve 6 g ammonium molybdate into 20 mL nanopure in a 100 mL volumetric
• If necessary, heat to 60°C until solution is clear, and allow to cool
• Dissolve 0.1455 g antimony potassium tartrate into solution
• Slowly add 70 mL sulfuric acid to solution, and allow to cool
• Dilute to final volume of 100 mL with nanopure
• Store in dark at 4°C

Ascorbic Acid Stock
• Dissolve 0.66 g ascorbic acid into nanopure into 5 mL of nanopure (use pipette to be totally accurate!)
• Neutralize with sodium bicarbonate and dispose down drain

Working Solution
• Prepare fresh daily!
• Add 2.5 mL acid molybdate stock into 80 mL nanopure in a 100 mL volumetric, mix well
• Add 1.0 mL ascorbic acid stock to solution
• Bring to final volume of 100 mL with nanopure
• Good for ~ 120 samples

Protocol to extract soil

1. Weigh out ~1.0 g of processed soil into 20 mL borosilicate tubes (I find it easiest to tare the test tube in a small beaker, and then use that to hold the test tube upright in the balance)
2. Add 10 mL of Mehlich 3 Extractant solution to each tube (make sure to include blanks!). It's easiest to do this using the pipette! Snip the ends off the pipette tip to make it easier (it won't affect the volume, but does allow for faster pipetting!).
3. Shake at 200 or more rpm for 5 min. at room temperature (I find it easiest to shake one tray at a time, but up to 5 can go on the shaker table)
4. Filter extracts gravimetrically through Whatman #1 filter paper (re-filter if extract is not clear). Here, set up a second set of test tubes in racks, making sure there is an open space between each test tube (e.g. a test tube rack with 4 rows of 10 columns will hold 10 test tubes. 2 rows of 5 columns.). Place a funnel directly into the top of the test tube, fold filter paper in, and pour your extract thru.

Protocol to run extracts

1. Into a 96 well tray, pipette 50 uL of extractant. (Make sure to do however many replicates you want! Either 3 or 4 for each sample).
2. Also make sure that you pipette in a standard curve using stock solutions. 0 – 100 ppm P is a good standard curve for soil.
3. Add 200 uL of working solution to each well. Tap the corners to mix. Allow for 10 minutes for colors to develop. Colors will be stable for ~2 hours.
4. Run the plates on the Epoch at 882 nm.

Calculating % P

1. Use the standard curve to calculate the ppm P in each filtrate sample.
2. To convert the ppm P in the filtrate to a "traditional" measure of ppm P (done using a standard soil scoop, etc) use the following equation:

ppm P in soil = calculated ppm P / wt (g) of soil used * 2 * 10

OR

lb/acre P in soil = calculated ppm P / wt (g) of soil used * 2 * 20

Reporting results:

"Available P was determined using the Mehlich 3 procedure (Frank *et al*. 2012)."