Modified Hedley fractionation protocol for sequential phosphorus extraction in soils

Purpose

Sequential phosphorus (P) fractionation, where the lability of soil (and manure) P is characterized based on solubility in a series of extractants is a useful method to study the effects of different variables on P transformations and availability. A fairly standardized designation is separation of extracted P into labile P (water-extractable and sodium bicarbonate-extractable), moderately labile P (sodium hydroxide-extractable, assumed to be associated with amorphous Al and Fe oxides and organic matter), stable inorganic P (hydrocholoric acid-extractable, assumed to be Ca-associated phosphates), and stable organic P (hydrocholoric acid + ashing extractable (Hedley et al. 1982; Ruttenberg 1992; Waldrip et al. 2015).

Chemicals

1. **0.5 M sodium bicarbonate** solution (42.00 g $NaHCO_3$/ L)
2. **0.1 M sodium hydroxide** solution (4.00 n NaOH/L)
3. **1.0 M hydrochloric acid** solution (In a volumetric flask, slowly add 82.1 mL concentrated HCl solution to ~300-500 mL ultrapure deionized water (DIW). Adjust the final volume of solution to 1000 mL with DIW.)

Protocol

Water-extractable P

1. Weigh out 1.0 g (± 0.05 g) of each soil sample into 50 mL centrifuge tubes. Record the exact weight of each tube and soil sample. Duplicate all samples.
2. Calibrate a bottletop dispenser or other dispenser and add 25mL of DIW to each sample.
3. Cap all tubes tightly and shake on an orbital shaker (250 rpm) 2 hours.
4. Centrifuge tubes at 4000 x g (about 6000 rpm on VWR benchtop centrifuges) for 30 minutes (remember to balance the rotor).
5. Decant supernatant and pass through a 0.45-mm nitrocellulose filter into a clean, labeled scintillation vial. If necessary, centrifuge sample a second time to remove more supernatant.
6. Leave ~10% of the volume of the vial empty to allow the sample to expand upon freezing by pouring off excess sample.
7. Weigh the tube with the soil sample and entrained solution.
8. Save soil residue and tube for the next step of the sequential extraction.

Sodium bicarbonate-extractable P

1. Calibrate a bottletop dispenser or other dispenser and add 25mL of **0.5M sodium bicarbonate solution** (pH 8.5) solution to each sample.
2. Cap all tubes tightly and shake on an orbital shaker (250 rpm) 16 hours.
3. Centrifuge tubes at 4000 x g (about 6000 rpm on VWR benchtop centrifuges) for 30 minutes (remember to balance the rotor).
4. Decant supernatant and pass through a 0.45-mm nitrocellulose filter into a clean, labeled scintillation vial. If necessary, centrifuge sample a second time to remove more supernatant.
5. Leave ~10% of the volume of the vial empty to allow the sample to expand upon freezing by pouring off excess sample.
6. Weigh the tube with the soil sample and entrained solution.
7. Save soil residue and tube for the next step of the sequential extraction.

Sodium hydroxide-extractable P

1. Calibrate a bottletop dispenser or other dispenser and add 25mL of **0.1 M sodium hydroxide solution** to each sample.
2. Cap all tubes tightly and shake on an orbital shaker (250 rpm) 16 hours.
3. Centrifuge tubes at 4000 x g (about 6000 rpm on VWR benchtop centrifuges) for 30 minutes (remember to balance the rotor).
4. Decant supernatant and pass through a 0.45-mm nitrocellulose filter into a clean, labeled scintillation vial. If necessary, centrifuge sample a second time to remove more supernatant.
5. Leave ~10% of the volume of the vial empty to allow the sample to expand upon freezing by pouring off excess sample.
6. Weigh the tube with the soil sample and entrained solution.
7. Save soil residue and tube for the next step of the sequential extraction.

Hydrochloric acid-extractable inorganic P

1. Calibrate a bottletop dispenser or other dispenser and add 25mL of **1M hydrochloric acid solution** to each sample.
2. Cap all tubes tightly and shake on an orbital shaker (250 rpm) 16 hours.
3. Centrifuge tubes at 4000 x g (about 6000 rpm on VWR benchtop centrifuges) for 30 minutes (remember to balance the rotor).
4. Decant supernatant and pass through a 0.45-mm nitrocellulose filter into a clean, labeled scintillation vial. If necessary, centrifuge sample a second time to remove more supernatant.
5. Leave ~10% of the volume of the vial empty to allow the sample to expand upon freezing by pouring off excess sample.
6. Weigh the tube with the soil sample and entrained solution.
7. Save soil residue and tube for the next step of the sequential extraction.

Hydrochloric acid-extractable organic P

1. Transfer each soil sample carefully to a small pre-weighed crucible.
2. Put samples in furnace and heat muffle furnace to 550°C.
3. Ash samples for 2 hours. Allow furnace to cool before removing samples.
4. Weigh samples three times in the crucible. Record all three weights.
5. Transfer soil sample back to its centrifuge tube.
6. Calibrate a bottletop dispenser or other dispenser and add 25mL of **1M hydrochloric acid solution** to each sample.
7. Cap all tubes tightly and shake on an orbital shaker (250 rpm) 16 hours.
8. Centrifuge tubes at 4000 x g (about 6000 rpm on VWR benchtop centrifuges) for 30 minutes (remember to balance the rotor).
9. Decant supernatant and pass through a 0.45-mm nitrocellulose filter into a clean, labeled scintillation vial. If necessary, centrifuge sample a second time to remove more supernatant.
10. Leave ~10% of the volume of the vial empty to allow the sample to expand upon freezing by pouring off excess sample.
11. Weigh the tube with the soil sample and entrained solution.

Colorimetric analysis

Analyze all samples for P on the LACHAT using the molybdate-blue procedure. Be sure to make all calibration standards in the extraction solution matrix. For more information on making standard curves see protocol on making a standard calibration curve. You do not need to filter

the standards through a filter. NOTE: Some samples will be very acidic and should not be run on the LACHAT without bringing up their pH. Attempting to run the samples without dilution or other pH adjustment will cause double refractory peaks to read out on the software and peak integration data values will not be reliable. Bring the samples up to a pH of ~1.2 by diluting them 3:13 (1 part sample to 13 parts total volume (add 10 parts water)). If very low P concentrations are anticipated, the sample pH may instead be adjusted by adding a small amount of concentrated sodium hydroxide solution to each sample.

References:

Hedley, M. J., J. W. B. Stewart, and B. S. Chauhan. 1982. Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations1. *Soil Science Society of America Journal* 46: 970. doi:10.2136/sssaj1982.03615995004600050017x.

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Waldrip, Heidi M., Paulo H. Pagliari, Zhongqi He, R. Daren Harmel, N. Andy Cole, and Mingchu Zhang. 2015. Legacy Phosphorus in Calcareous Soils: Effects of Long-Term Poultry Litter Application. *Soil Science Society of America Journal* 79: 1601. doi:10.2136/sssaj2015.03.0090.