Protocol for soil organic P extraction (sodium bicarbonate + digestion)

Purpose

This procedure is a shortened version of the Hedley fractionation (Hedley 1982) to extract non-occluded inorganic phosphorus (P_i) from soil. The extract is then digested in potassium persulfate and re-analyzed for total P (TP). The difference between the TP and P_i is the organic fraction (P_o).

Chemicals

1. Sodium bicarbonate ($NaHCO_3$)
2. Sodium hydroxide (NaOH)
3. D Fructose 6-phosphate salt ($C_6H_{11}O_9PNa_2$-P/L, M.W. 304.1g/mol)
4. Sulfuric acid (H_2SO_4)
5. Potassium persulfate ($K_2S_2O_8$, M.W. 270.3 g/mol)

Bicarbonate solution

1. Fill a 2L volumetric flask half way with DIW water
2. Weigh out 84g of **sodium bicarbonate ($NaHCO_3$)**
3. Weigh out 1g of **sodium hydroxide (NaOH)**
4. Add these to the volumetric flask (add a little $NaHCO_3$ at a time and stir) and stir until dissolved and bring to volume.
5. Fill the flask to 2L with DIW water
6. Pour the 2L of bicarbonate solution into a bottle, attach bottletop dispenser to the top (calibrate dispenser for 30ml with DIW)

Inorganic phosphorus (P_i) extraction protocol

1. Oven-dry soil at 60ºC for 24 hours.
2. Weigh 3 g of soil into acid or base washed (and labeled) centrifuge tubes*
3. Add 30 mL of bicarbonate solution to each tube.
4. Shake centrifuge tubes for **19** hrs.
5. Filter extract through Whatman No. 42 or Fisherbrand Q2 paper into acid or base washed, labeled scintillation vials.
6. Store extracts in vials in the freezer until analysis.

* Make sure to include replicates at least every 10 samples as well as blanks

Colorimetric analysis

Analyze P_i on the LACHAT using the molybdate-blue procedure. Be sure to make all calibration standards in the bicarbonate solution matrix.

Total phosphorus protocol (persulfate digestion)

Digested standards

In order to determine the recovery of P_o, you must make two calibration curves: one with P_i and the other with P_o. To make the organic P stock standard calibrant of 1000 mg $C_6H_{11}O_9PNa_2$-P/L (**D Fructose 6-phosphate salt**, M.W. 304.1g/mol): add 0.9817 mg of $C_6H_{11}O_9PNa_2$ (to 100mL of DIW and dissolve. Make 2 large calibration curves (6-7 standards each) for P_o and P_i in the bicarbonate 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.

Digestion procedure

1. **The day before:** Make 0.9 *M* **sulfuric acid** (H_2SO_4) by filling a 1L volumetric flask with ~800mL of DIW, add 50mL of concentrated H_2SO_4, and let cool overnight. (Make as much as you need: 10mL per tube)
2. Top off the volumetric flask to 1L with DIW.
3. Weigh out 0.5g of **potassium persulfate** onto weigh paper and pour this amount very carefully into each tube.
4. Using DIW calibrate a pipette or other dispenser to 5 mL.
5. Write down exact amount and pipette 5 mL of bicarbonate sample and the etched digestion tube number that the same was pipetted into. Place tube on vortexer until potassium persulfate is dissolved.
6. Include the blanks, replicates and calibrants. Weigh 5mL of standards (inorganic and organic) and blanks into digestion tubes.
7. Fill a beaker with some of the 0.9 *M* H_2SO_4
8. **In the hood:** Add 10 mL 0.9 *M* H_2SO_4 1 mL at a time with a calibrated pipette or other dispenser, then cap loosely. This is a reactive process, so add as slowly as you need to in order to avoid boiling over.
9. Cap tightly and place in autoclave (with a slow-exhaust on it) for 60 minutes (20 minutes of slow exhaust). Make sure the autoclave gets up to the appropriate temperature and pressure before starting to time the run.
10. Open autoclave door and allow samples to cool until they can be comfortably handled.
11. Pour into acid or base washed (and labeled) scintillation vials.
12. Run for TP on LACHAT using the molybdate-blue procedure. NOTE: The 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 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). Run a P_i curve created from inorganic P standards created in DIW. This curve should not be diluted and standards should be run as calibration standards. Run two reps of each calibration standard as unknown samples – the digested inorganic and organic P standards that were created in the bicarbonate matrix. (This is three total standard curves). Run a sample rep every 10 samples.
13. Compare your concentration of your bicarbonate P_o curve to your P_i curve to determine the percent recovery of P_o from the digestion process. The digestion will not convert 100% of the P_o to P_i so the sample P_o value will have to be back calculated by comparing the standard curves.
14. Back calculate the dilution of bicarbonate sample (To obtain the accurate concentration value of your original samples, remember that the sample was first diluted with H_2SO_4 and then again with DIW).

Colorimetric analysis

Analyze TP on the LACHAT using the molybdate-blue procedure.

Calculations

Organic P is equal to the difference between total P and inorganic-P (Eq 1) $P_o=TP-P_i$

References:

Hedley, M. J., Stewart, J. W. B. & Chauhan, B. S. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970–976 (1982).