Hydrogen Peroxide-Nitric Acid (KMD) Digestion

From Kurth et al. 2006 (in *Geoderma*) and Pingree 2011 (MS thesis)
Updated May 2019 by Lauren Hendricks

Wear gloves!!

Procedure

1. Dry samples for 24 hours at 60ºC
2. Grind several grams of soil to ~1µm with the ball mill. 8 minutes at 1500rpm was usually sufficient, but some samples needed more time. **(S, g)**
    a. Keep both samples and ball mill cups/balls were as dry as possible (e.g., in oven immediately before putting into ball mill) to make it easier to get more of the sample out of the cups.
    b. This can be done well in advance of the following steps. Store ground soil in plastic bags.
3. Put ~0.85 g of ground soil in a 125mL Erlenmeyer flask. [Note that this is modified from the original procedure, which called for ~1g of soil in a 50mL flask. Reduced mass of soil used AND increased flask volume to eliminate problems with samples boiling over once chemicals added and placed in bath.]
4. Add 20 mL of 30% H_2O_2 and 10 mL of 1M HNO_3 to Erlenmeyer flask
    buy 30% H_2O_2 at strength (stocked in Chem Stores)
    make 1.5L of 1M HNO_3 by adding 97.14mL of stock HNO_3 to 375 mL of water, and then dilute to 1500mL (in a volumetric flask). To make 1L of 1M HNO_3 add 64.8mL of stock HNO_3 to 250 mL of water, and then dilute to 1000mL (in a volumetric flask).
        i. Remember to always add acid to water!!!
        ii. Store 1M HNO_3 in glass bottle.
        iii. Stock HNO_3 is 15.442M
5. Mix for 30 minutes at room temperature.
    a. Not necessary to use stir bar/plate; can just swirl occasionally.
6. Loosely cover flask with aluminum foil. Red wires are useful to hold the foil onto the flask, but make sure there is some air exchange in the flasks (so they don't explode).
7. Weigh (and record weight of) filter papers.
    a. Use glass filter papers if planning on radiocarbon dating (e.g., Whatman GF/C).
    b. If only quantifying pyrogenic C/N, paper filters should be okay (though there is a possibility of paper artificially inflating C values).
    c. Whatman 1 qualitative filters with diameter of 110mm seem to work well .
8. Heat flask to 90ºC for 16 hours in water bath (in fume hood)., but also be careful that the samples don't boil over or lose too much liquid.

START	END (+ 16 hours)
8:00 AM	12:00 AM
9:00 AM	1:00 AM

10:00 AM	2:00 AM
11:00 AM	3:00 AM
12:00 PM	4:00 AM
1:00 PM	5:00 AM
2:00 PM	6:00 AM
3:00 PM	7:00 AM
4:00 PM	8:00 AM
5:00 PM	9:00 AM
6:00 PM	10:00 AM
7:00 PM	11:00 AM
8:00 PM	12:00 PM
9:00 PM	1:00 PM
10:00 PM	2:00 PM
11:00 PM	3:00 PM
12:00 AM	4:00 PM
1:00 AM	5:00 PM
2:00 AM	6:00 PM
3:00 AM	7:00 PM

9. Let cool.
10. Filter through pre-weighed filter paper (in fume hood).
    a. Use small plastic funnels.
    b. May take several rinses with dH_2O to get all of the sample out of the flask.
    c. Leave to drip into original flasks for minimum of several hours.
    d. Dispose of acid-peroxide in waste containers.
11. Dry filters for 24 hours at 60ºC
12. Weigh (and record weight of) dried filter papers + sample. Subtract out weight of filter paper = Mass of digested soil: **(R, g)**
13. Grind residual material using a mortar and pestle until it is homogenous [note that getting the sample off of the filter paper can be challenging]
14. Measure in elemental analyzer **(P, %)**

In each batch, run standards with known amount of pyrogenic material and organic matter, made with organic matter free matrix.

Calculations

g PyC / g soil = P * R / S

Sample Workflow

Batch	Weigh Samples	Label Bags & Weigh Filters	Into Bath/Out of Bath (16 hours)	Filter*	Into Drying Oven	Weigh Dried Filters + Sample
example 1	afternoon of day 1	afternoon of day 1	afternoon of day 1 (~4-5pm)/day 2 (~8-9am, depending on when digestion started)	morning of day 2	morning of day 3	morning of day 4
example 2	afternoon of day 2	afternoon of day 2	afternoon of day 2(~4-5pm)/day 3 (~8-9am, depending on when digestion started)	morning of day 3	morning of day 4	morning of day 5
example 3	afternoon of day 3	afternoon of day 3	afternoon of day 3 (~4-5pm)/day 4 (~8-9am, depending on when digestion started)	morning of day 4	morning of day 5	morning of day 6

*If only two sets of Erlenmeyer flasks are available, right after taking samples (e.g., batch 2) out of water bath, transfer filters to drying oven (e.g., batch 1) and immediately wash flasks from the previous batch (e.g., batch 1) so they can dry several hours before adding new sample (e.g., batch 3)

Other Notes

- The residual after the KMD represents the black carbon; the difference between before and after the digestion is the non-black carbon.
- Look at Maestrini and Miesel 2017 (in *Organic Geochemistry*) for a modification that accommodates organic matrices. This would be used for O horizon samples.

References

Kurth, V. J., M. D. MacKenzie, and T. H. DeLuca. 2006. Estimating charcoal content in forest mineral soils. Geoderma 137:135–139.

Pingree, M. R. A. 2017, May 16. Fire, Charcoal, and the Biogeochemistry of Carbon and Nitrogen in Pacific Northwest Forest Soils. Thesis. University of Washington.

Pingree, M. R. A., P. S. Homann, B. Morrissette, and R. Darbyshire. 2012. Long and Short-Term Effects of Fire on Soil Charcoal of a Conifer Forest in Southwest Oregon. Forests 3:353–369.

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