

Parr Dry Ash Procedure: Phytolith Extraction Laboratory Report

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Date: November 15, 2011

Goal

Extraction of phytoliths from dried specimens of *Schinus molle* (Anacardiaceae), stem and leaf using dry ashing method described in Parr et. al 2001.

Procedure

The procedure outlined in Parr et. al 2001 for dry ash extraction method is as follows, with my own notes attached:

- (1) Weigh approximately 0.2 g of dried plant material and record (*I did not have 0.2 g of dried plant material available, with .0684 g of stem material and .0054 g of leaf material*)
- (2) Rinse plant material in dH₂O and transfer to crucible.
- (3) Transfer crucible to muffle oven.
- (4) Heat to 500°C and hold for eight hours (overnight)
- (5) Remove crucibles and transfer contents to test tubes.
- (6) Add 10 ml of 10% HCl to test tube—heat in water bath at 70°C for 20 min or until reaction stopped (*following the advice of Rob Cuthrell, instead of using 10 ml of a diluted 10% solution of HCl, I used a drop of HCl at the concentration listed on the bottle and placed the test tubes in a bath of water that had been heated to near boiling in a microwave. The test tubes were kept in the water until reactions stopped*).
- (7) Centrifuge at 3500 rpm for 5 min and decant.
- (8) Rinse with dH₂O and centrifuge at 3500 rpm for 5 min and decant.
- (9) Add 10 ml of 15% H₂O₂—heat in water bath at 70°C for 20 min or until reaction stops (*following the advice of Rob Cuthrell, instead of using 10 ml of a diluted 15% solution of H₂O₂, I used a drop of H₂O₂ at the concentration listed on the bottle and placed the test tubes in a bath of water that had been heated to near boiling in a microwave. The test tubes were kept in the water until reactions stopped*).
- (10) Centrifuge at 3500 rpm for 5 min and decant.
- (11) Rinse with 10 ml of dH₂O and centrifuge at 3500 rpm for 5 min and decant. Repeat rinse.
- (12) Add 1 ml of 100% ETOH and leave overnight to dry (*Since we did not have ethanol on hand, I used 1 ml of methanol instead and left the samples to dry for a week. Acetone was also posited as a possible substitute. Upon my return, the samples had not dried. To remedy this, the samples were placed in the oven at 80°C which proved very effective. The samples dried within a few hours*).
- (13) Weigh dried material, calculate phytolith weight and transfer to labeled vials with as little 100% ETOH as possible (*My samples were completely dried when weighed. Final sample weight was calculated to be 16 mg for the stem sample and 2.6 mg for the leaf sample*).

(14) Mount using Benzyl Benzoate (*I used immersion oil described in the section below*).

Mounting

Mounting of the recovered phytolith material was achieved with immersion oil using the method described by Rob Cuthrell's "Phytolith Sample Collection and Processing Procedure" (2011). For each 1 mg of phytolith extract, 0.05 (0.046 g) of Cargille "Type B" immersion oil was added to each sample, producing a standardized density phytolith extract in each sample. Then, using a round toothpick, each sample tube was stirred vigorously for approximately 1 min to homogenize the material. A small portion of the phytolith extract was then drawn into a disposable glass pipet and one drop was expelled onto the center of a glass slide. Using a round toothpick, the material was mixed and spread over a rectangle of roughly 20x30 mm in size. The toothpick was used to pop any bubbles that formed during mixing, and the extract was covered with a 24x30 mm cover slip. Fast drying, clear fingernail polish was then used to seal the cover slip right before any of the phytolith mixtures reached the edge. Slides were then dried overnight and are ready for viewing.

Results

Phytoliths were mounted on slides and viewed on the BX-51 microscope. Unfortunately, the aforementioned process did not yield any identifiable phytoliths. It should be noted that at this time, it is unknown how prevalent phytoliths are in the family Anacardiaceae, though Dorian Fuller's phytolith website indicates they are at least present in *Pistachia* fruit. In hindsight, it probably would have been more useful to process material from taxa known to produce phytoliths. In the future, it would be interesting to compare the dry ashing method for herbarium specimens to the microwave digestion method also posited by Parr using taxa that yield a prolific amount of phytoliths.

Works Cited

Cuthrell, R. 2011. Phytolith Sample Collection and Processing Procedure. McCown Archaeobotany Laboratory Report #67.

Parr, J.F. et al. 2001. A microwave digestion method for the extraction of phytoliths from herbarium specimens. *Review of Paleobotany and Palynology* 116: 203-212.

Dorian Fuller's Old World Reference Phytoliths version 1.3; Accessed November 15, 2011. Available at: <http://www.homepages.ucl.ac.uk/~tcrndfu/phytoliths.html>