

Phytolith Reference Collection Processing - Dry Ashing

This protocol is designed to clean, dry ash, wash with acid, and mount modern plant specimens as onto slides as phytolith reference comparisons. The procedure is adapted from Pearsall (2015: 294) and Piperno (2006: 97) and takes approx. 4 days from start to finish.

PRETREATMENT OF PLANT MATERIALS ~ 20 mins + overnight

1. Cut and weigh samples, then place in labeled centrifuge tubes (15 to 50 ml tubes). A sample size of 0.1 g is usually sufficient, however, we have been processing in excess of a gram and keeping a collection of ash.
2. Fill the tubes with 2 percent detergent solution, cover, and let sit overnight.

CLEANING AND RINSING ~ 30 mins + overnight

3. Place test tubes in an ultrasonic bath (sonicator) for 15 minutes.
4. Remove test tubes from the bath and rinse each sample with DI water.
5. Allow samples to dry before processing. Place in the desiccator at 30°C to dry overnight.

DRY ASHING AND CHEMICAL TREATMENT ~ 8 hrs + overnight

1. Using sterilized scissors or a mortar and pestle, crush, grind, and/or cut samples to small pieces (0.5 cm x 0.5 cm).
2. Place material in a ceramic crucible and ash for approx. 6 hrs at 450°C in the muffle furnace (Pearsall's samples have been done as quickly as 2 hrs; samples are finished when the residue is whitish-grey in colour). The smaller the particle size, the less time it will take in the oven.
3. Once cooled to room temperature, transfer ash to 1.5 ml microcentrifuge tubes for chemical treatment and/or for untreated ash storage (if so desired: our lab likes to have excess ash in case we want additional samples).
4. Fill microcentrifuge tube with 1N HCl. Centrifuge at 6000 rpm for 5 mins, decant supernatant, and fill with DI water. Repeat washing with DI water three times total.
5. Place samples in the desiccator at 30°C to dry overnight.

MOUNTING ~ 30 mins for 8 samples

1. Using a glass pipette (wiped clean with acetone or isopropyl alcohol), place a dime-sized amount of Cargille immersion oil B (or mounting agent of choice) on the slide.
2. Using a microspatula (wiped clean with acetone), remove approx. 0.1 g of material and shake (gently) onto slide. Mix phytolith material with oil to evenly disperse phytoliths throughout the slide. Periodically, make and examine a test slide of mounting agent to check for contamination.
3. Place a cover slip (22mmx22mm or 25mmx25mm) over the oil and let it spread evenly. Write your sample number and description onto the slide. After all slides have been mounted, paint the edges of the cover slip with nail polish to seal.

Revision History:

Created by Kali Wade, Boston University: 11/1/16

Standardized by Kali Wade, Boston University: 2/22/18

References:

Pearsall, Deborah.

2015. *Paleoethnobotany, Third Edition: A Handbook of Procedures*. Left Coast Press.

Piperno, Dolores R.

2006. *Phytoliths: a comprehensive guide for archaeologists and paleoecologists*. AltaMira Press, Lanham (Md.)