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Phytolith Reference Collection Processing - Dry Ashing

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Guidelines

This protocol includes the use of hazardous chemicals and therefore, ensure you are using any and all personal protective equipment possible. At minimum, I would recommend the use of a fume-hood when dealing with chemicals and PPE that includes a labcoat, goggles, gloves, and hair tied back securely.

Troubleshooting

Fail-Safe Practices

1 Wipe down all equipment, surfaces, and tools used with soap and water followed by acetone (beginning and end of every day of the project).

Pretreatment of Plant Materials

2 Cut and weigh samples, then place in labeled centrifuge tubes (15 mL 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.

3 Fill the tubes with [M] 2 % volume detergent solution (Liquinox) cover, and let sit overnight.

Cleaning and Rinsing

15m

4 Place test tubes in an ultrasonic bath (sonicator) for 00:15:00 15m

5 Remove test tubes from the bath and rinse each sample with DI water.

6 Allow samples to dry before processing. Place in the desiccator at 30 °C to dry overnight.

Dry Ashing and Chemical Treatment

7 Using sterilized scissors or a mortar and pestle, crush, grind, and/or cut samples to small pieces (0.5 cms x 0.5 cms).

8 Place material in a ceramic crucible and ash for approx. 06:00:00 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. 6h

9 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).

10 Fill microcentrifuge tube with [M] 1 Molarity (M) . Centrifuge at 6000 rpm, 00:05:00 decant supernatant, and fill with DI water. Repeat washing with DI water three times total. 5m

11 Place samples in the desiccator at 30 °C o dry overnight.

Mounting onto Microscopy Slides

12 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.

13 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.

14 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.

Protocol references

Revision History:
Created by Kali Wade, 28/10/2020, see Boston University Research Protocols:
https://sites.bu.edu/ealab/collections/research-protocols/

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.)

Ulethbridge Lab Notes

Acid Rinse – Add couple drops 5% HCL to
crucible for transferring into tubes
Wash hands and wipe down lab coat with wet
hands to reduce static
If crucible with ashed sample is extremely
static, use a damp papertowel to wipe around outside of crucible, wipe down end
of spatula and around the outside of the test tube to reduce the static,
thereby helping in the transfer of the sample from the crucible to the
test-tube

3 times of 500ml HCL for removing sample from
crucible into test tube, also ensures test tubes are balanced for centrifuge

Write scientific name, common name and plant
part on test tube

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