Phytolith Reference Collection Processing - Wet Ashing

This protocol is an alternative method to processing modern plant material for phytolith reference collections, using nitric acid to remove organic material instead of burning in a muffle furnace. If one is concerned about altering phytolith morphologies due to high temperatures, acid washing offers an alternative to avoid this problem and, as muffle furnaces are generally restricted to the number of cribubles that can fit, one may be able to process more samples simultaneously (Pipero 2006; 97). This procedure will take approximately 2.5 hrs, over 2 days, to produce 4 samples.

SAMPLE PREPARATION ~ 2 mins

1. Place a small amount of modern plant material (1 cm^2 is usually plenty) into a 15 ml glass centrifuge tube and label appropriately.

REMOVING ORGANICS ~ 1 hr

1. Add 5 ml of concentrated nitric acid to the tube and carefully monitor any reaction.
2. If no reaction, add another 5 ml of concentrated nitric acid, and repeat up to a total of 15 ml of nitric acid in the tube.
3. Place tubes in a beaker and fill the beaker with water to an equivalent level of the nitric acid in the centrifuge tubes.
4. Place the beaker on a hot plate and increase the heat to have the water just simmering.
5. Using glass rods, stir constantly.
6. If there is little reaction, add a small amount (1/4 tsp.) of potassium chloride ($KClO_3$) to each tube.
7. Continue simmering, adding small amounts of potassium chloride periodically and stirring frequently.
8. Wait until liquid turns from a red or dark yellow color to a clear yellow color, and virtually no reaction is noted during stirring (i.e., almost no bubbling at surface of supernatant)

RINSING CHEMICAL ~ 20 mins + overnight

1. Rinse samples three times total*.
2. Place in dessicator to dry sample overnight.

MOUNTING MICROSCOPE SLIDES ~ 30 mins

1. Using a glass pipette, place 3-4 drops of Cargille Immersion oil onto slide.
2. Weigh slide and tare.
3. Place phytolith material onto the slide using a sterilized microspatula, and record weight.

4. Mix the oil and phytolith material with microspatula to evenly disperse material across the slide.
5. Gently drop 25 x 25mm coverslip onto slide and let oil disperse to the sides and corners.
6. Seal slide and coverslip with clear nail polish.

*To Rinse Samples: Fill tube with DI water. Vortex for 5 sec. Centrifuge for 5 min at 1000 rpm. Remove supernatant with pipette and discard.

Revision History:
Created by Stephanie Simms, Boston University: 9/18/13
Standardized by Kali Wade, Boston University: 3/8/18

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