Phytolith Processing – Modified for Clay-Rich Sediments

This protocol is modified from Lombardo et al. (2016) sonication and Lisa-Marie Shillito's (2011) methods, best for samples that are clay-rich. The frequent use of sonication should produce cleaner slides however, may disarticulate multicellular phytolith aggregates. Processing time: approx. 1 week to process 8 samples.

SAMPLE PREPARATION (optional) ~ 3 hrs
1. Sterilize/Desiccate if necessary (international soils).
2. Sieve through a 250µm mesh.

INITIAL CLAY REMOVAL ~ 8-10 hrs
1. Weigh 10 grams of sediment into a clean 400 ml glass beaker.
2. Add 15 mls of 5% sodium hexametaphosphate and DI water to a total volume of 350 ml. Stir the samples and let them sit for 60 min (allowing phytoliths to settle to the bottom of the beaker as clay remains suspended in the water nearer the surface).
3. Pour off supernatant to a total of 100 mls and refill the beaker to 350 mls with DI water.
4. Stir and let settle for 60 min.
5. Decant again. Repeat until samples are fairly clear. Place filter paper on top of the beakers between sitting to avoid contamination.
6. Transfer samples to crucibles, desiccate overnight.

INITIAL CLAY REMOVAL ~ 2 hrs
1. Place samples in the muffle furnace and heat at 550 °C for 2 hrs. Allow to cool overnight.

REMOVING CARBONATES ~ 1 hr
1. Transfer samples to 50 ml centrifuge tubes.
2. Add 5 mls of 10% HCl acid to the tubes (slowly) until the samples stop fizzing after shaking.
3. Add DI water to the 40 ml line of the tubes. Vortex for 5 sec.
4. Centrifuge 2 mins at 2500 rpm.
5. Decant supernatant.
4. Rinse* samples 3 times.

REMOVING ORGANICS ~ 2 hrs
1. Add 20 ml of 30% hydrogen peroxide (slowly; monitor and manage any reaction).
2. Place in ultrasound bath for 30 min, set to "On" and 30 min set to "Off", until no reaction is observed.
2. Rinse* samples 4 times.

REMOVING CLAY ~ 2-3 hrs

1. Fill samples with 45 ml of 5% sodium hexametaphosphate. Vortex for 5 sec.
2. Place in ultrasound bath for 10 min. Vortex for 5 sec.
3. Centrifuge for 3 min at 1500 rpm.
4. Remove supernatant with pipette.
5. Repeat steps 1-4 until supernatant is clear (to a maximum of 10 rinses).
6. Rinse* samples 4 times.

REMOVING HEAVY MATTER ~ 1.5 hrs

1. Add 15 ml of sodium polytungstate at 2.3 specific gravity to the samples. Centrifuge samples at 3000 rpm for 5 min.
2. Transfer 10 ml of SPT with floating fraction to a second tube and repeat this process twice (three times TOTAL, the new tube should contain 30 ml of supernatant).
3. Fill the second tube with DI water, centrifuge at 3000 rpm for 10 min.
4. Recover the diluted SPT to an addition tube for recycling later.
5. Rinse* the samples 4 times.
6. Dessicate overnight.

MOUNTING MICROSCOPE SLIDES ~ 2 hrs

1. Using a glass pipette, place three- four drops of Cargille Immersion oil onto slide.
2. Weigh slide and tare.
3. Place 2 mg of phytolith material onto slide using a sterilized microspatula, record weight of phytolith material on slide.
4. Mix the oil and phytolith material with microspatula to evenly disperse material across the slide.
5. 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 to 40ml line with DI water. Vortex for 5 sec. Centrifuge for 2 min at 2500 rpm. Remove supernatant with pipette and discard.

Revision History:

Created by Kali Wade and Sydney Hunter, Boston University: 1/11/18

References:

Lombardo, Umberto, Javier Ruiz-Pérez, and Marco Madella
2016 Sonication improves the efficiency, efficacy and safety of phytolith extraction. *Review of Palaeobotany and Palynology* 235: 1–5

Shillito, Lisa-Marie
2011 *Daily activities, diet and resource use at Neolithic Çatalhöyük: microstratigraphic and biomolecular evidence from middens*. Archaeopress, Oxford.