Phytolith Processing – Standard Protocol

This protocol is a standard phytolith processing method, designed to produce permanent slides for soils of average or unknown clay, organic, and carbonate content. Advantages of this protocol include gravity sedimentation and burning in a furnace to clean the samples thoroughly, with minimal use of hazardous chemicals. A disadvantage of this method is its lack of sediment fractionation, leaving multicell aggregates, small phytolith bodies, and diatoms together in the same sample. Processing time: approx. 1 week to process 8 samples. This protocol is based on White (2013), Piperno (2006), and Jenkins et al. (2011) methods.

SAMPLE PREPARATION ~ 1-2 hrs

1. Dry the sediment samples in a drying oven overnight.
2. Break up sediment clumps with an agate mortar and pestle.
3. Sieve sample through a 250 µm mesh.
4. Weigh about one gram of sediment (between 800-1500 mg depending on phytolith content) in a 50 ml centrifuge tube (one can use between 5-50 g sediment).
5. Weigh 8 samples.

REMOVING CARBONATES ~ 1 hr

1. Add 10% HCl acid to the tubes (slowly) until the samples stop fizzing.
2. Add DI water to the 40 ml line of the tubes.
3. Centrifuge for 5 min at 2000 rpm.
4. Decant water/HCl and keep pellet in bottom of tube.
5. Add more DI water to 40 ml line and mix using Vortex machine.
6. Centrifuge again for 5 min at 2000 rpm.
7. Repeat steps 4,5, and 6 once more.

REMOVING CLAYS ~ 8-10 hrs

1. Add 15-20 ml of 5% sodium hexametaphosphate to each centrifuge tube.
2. Mix vigorously using Vortex machine for 30 sec. Pellet must be dispersed.
3. Pour into 400 ml beaker, rinse tube, and fill beaker to 275 ml line with DI water.
4. Stir and let set for 70 min.
5. Pour off supernatant steadily, leaving 150 ml in beaker.
6. Add DI water to the 275 ml line.
7. Stir and let set for 60 min.
8. Pour off supernatant and refill to 275 ml line.
9. Repeat steps 6,7, and 8 between four to six times, depending on sample.
10. Last pour: leave just 50 ml in beaker.
11. Pour sample into crucible. Once settled, pipette off excess water or evaporate using drying rack/heat lamp.

REMOVING ORGANIC MATTER ~ 3 hrs

1. Crush sample in crucible to fine powder to ensure it is dry.
2. Place in muffle furnace for 2 hrs at 500°C.

REMOVING HEAVY MATTER ~ 3 hrs

1. Pour 3 ml of 2.3 specific gravtiy sodium polytungstate into 8 15 ml centrifuge tubes.
2. Add sample. Vortex for 5 sec.
4. Move directly to centrifuge and be sure it is balanced. Centrifuge for 10 min at 800 rpm.
5. Pipette supernatant into a new set of 15 ml centrifuge tubes. Discard pellet.
6. Add DI water to 11 ml line and vortex for 5 sec.
7. Centrifuge for 5 min at 2000 rpm.
8. Keep pellet at bottom and discard supernatant for SPT recycling.
9. Repeat steps 6, 7, and 8 at least three times.
10. Weigh 10 ml beaker and flick phytolith pellet into it. Use DI water to clean centrifuge tube completely of phytoliths.
11. Dry beaker contents using heat lamp or drying cabinet.

MOUNTING MICROSCOPE SLIDES ~ 2 hrs

1. Weigh precleaned slide and add 2 mg of phytolith material.
2. Using pipette, place nine drops of Entellan New Mounting Media onto slide.
3. Mix Entellan and phytoliths evenly and add coverslip.
4. Leave to dry for one week.

Revision History:

Created by Chantel White, Boston University: 11/19/06
Standardized by Kali Wade, Boston University: 1/17/18

References:

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