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

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1

Modified Rapid Phytolith Methods

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Kali R Wade: This would not be possible without the work of Ofir Katz and colleagues (2010) and additional modifications from Rosa Maria Albert and colleagues (pers comm.)



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ABSTRACT

Processing procedures here are modified from those outlined in Katz et al 2010. Samples can be processed in this way and, following completion, rinsed with deionized water and dried to a stable state for storage and to make extra slides for morphological IDs.

SAFETY WARNINGS

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 the use of a labcoat, goggles, gloves, and hair tied back securely.

BEFORE START INSTRUCTIONS

Follow the "fail-safe" practices outlined here, in addition to any other health and safety paperwork or contamination recommendations from your organization.

Fail-Safe Practices

30m

Wiping down all equipment, surfaces, and tools used with soap and water followed by acetone

(beginning and end of every day of the project).

- 2 Checking and adjusting the density of sodium polytungstate (SPT) to conform to the protocol being used (normally between 2.3 and 2.5 g/ml).
- Plating one slide of each hydrocloric acid (HCl) SPT, and the mounting agent (likely Cargille Immersion Oil, Type B) and viewing them under microscopy to ensure no contamination.
- 4 Calibrating analytical balances.

Sample Preparation

- 5 If necessary, place samples in desiccator overnight, set to 30°C.
- 6 Label 1.5 ml centrifuge tubes (2 per sample being processed) with sample ID.
- 7 Sieve samples through 0.5 mm mesh, rinsing mesh between each sample with soap and water, acetone, then deionized water.
- **8** Weigh 30-80 mg of sediment into 1.5 ml centrifuge tube. Ensure balance is tared following the weight of the tube, prior to weighing sediment, with balances' scale and lab doors closed.

Organic and Carbonate Removal

8m

- 9 Add 50µl of 6N HCl, waiting for bubbling to cease (give the samples a few minutes). Shake tube to ensure chemical gets through all of the sample
- **10** Add 450μl of 2.5 g/ml SPT

Breaking up Soil Aggregates & Creating Pellet

22m

11 Vortex for 3 sec, sonicate for 10 min, vortex 3 sec

11m

12 Centrifuge for 10 min at 6rpm

11m

Plating Slides

- Remove supernatant to clean 1.5ml test tube. Be mindful of proper labels.
- 14 Vortex supernatant for 3sec
- 15 Pipette 50µl of supernatant onto a labelled microscope slide and cover with a coverslip. Seal with nail polish. Slides last between 6-12 hours before crystallization