A Comparison of Two Phytolith Extraction Techniques for Archaeological Sediment Samples

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Objective:

Comparing the 'hot water bath' (Lentfer & Boyd 1999; Piperno 2006:92) and 'microwave' (Cuthrell 2011; Parr 2001) techniques for phytolith extraction from the archaeological sediments using sediment from the archaeological site of Ubaté, Colombia at the test. The 'hot water bath' technique provides a low-tech and less costly method of extracting phytoliths, requiring only boiling water. On the other hand, the microwave method presents a faster although much more expensive method of phytolith extraction from soils (Parr 2001). The purpose of this comparison was to determine whether these two phytolith extraction techniques could be used interchangably and produce easily comparable data, thus opening up their use in different research settings. I started out with one preparation procedure and then separated the sample only at the digestion stage (V), after that, samples from both procedures were processed in the same manner. This research was completed in the autumn of 2011 in the Archaeological Research Facilities wet laboratory, which is set up for all micro botanical processing.

I. Sample Preparation:

1. Sediment samples (of at least 10 grams) were heated at 120° for 20 hours to dry and decontaminate samples according to USDA regulations (United States Department of Agriculture, Animal and Plant Health Inspection Service [USDA SCS] 2011: 4).
2. Dried sediment was then gently ground into a fine powder in a mortar and pestle and screened through a 500µ geological sieve.

II. Deflocculation:

1. Approximately 10 g of this fine sediment from each sample was transferred to a 50 ml polypropylene centrifuge tube and mixed with 30 ml of a 10% bicarbonate solution.
2. Tubes were vortexed and were placed horizontally in a test tube shaker and left overnight to deflocculate (at least 8 hours).
3. Samples were removed from shaker, centrifuged for 5 minutes at 3000 rpm. Supernatant was discarded leaving about 2 cm of the supernatant behind.
4. Approximately 40 ml of distilled water was added to each tube, the tube was vortexed again and centrifuged for 5 minutes at 3000 rpm. The supernatant containing diluted bicarbonate solution was discarded.

III. Clay Removal:

1. The tube was filled to '45 ml' line with distilled water, samples were vortexed and centrifuged for 75 seconds at 1000 rpm.
2. Using a 20 ml syringe (with no needle) the supernatant was removed until the water column was about 2 cm above the pellet.
3. Steps 1-2 were repeated until the supernatant was no longer cloudy.

IV. Sample Drying before Chemical Processing

1. Samples were transferred to 50 ml beakers and heated in a low-temperature oven at 60°C for 22 hours.
2. After drying, the pellet at the bottom of the beaker was gently broken up using a glass-stirring rod and any material adhering to the sides of the beaker was released using the glass rod.

Two different digestion techniques were used, splitting each archaeological sample, to be able to compare their results: Technique 1. The hot water bath digestion and Technique 2. Microwave digestion.

Digestion Techniques

V. Technique 1: Hot Water Bath Digestion

1. Dry sediment was mixed with 3 mL of hydrochloric acid (HCl) in 50 ml polypropylene tubes, placed in a 70° hot water bath, and periodically stirred until reaction ceased.
2. Samples were filled with distilled water to the '45 ml' line, vortexed, and centrifuged for 2 minutes at 3000 rpm at which point samples were gently tilted from side to side to assure that any adhering material would be released into the supernatant. Then, they were centrifuged for another 5 minutes at 3000 rpm and the supernatant was discarded.
3. Step 2 was repeated.
4. Samples were mixed with 5 ml of nitric acid (NNO_3), placed in 70° hot water bath, and periodically stirred until reaction subsided.
5. Samples were mixed with 2 ml of hydrogen peroxide (H_2O_2). Additional small amounts of H_2O_2 were periodically added until no reaction with H_2O_2 addition was observed (these particular samples required at maximum a total of 18 ml of H_2O_2 per sample).
6. Samples were filled with distilled water to the '45 ml' line, vortexed, and centrifuged for 2 minutes at 3000rpm. Samples were gently tilted from side to side to assure that any adhering material would be released into the supernatant. They were centrifuged for another 5 minutes at 3000 rpm and the supernatant was then discarded.
7. Step 6 was then repeated two times to remove any residual chemicals.
8. Samples were transferred to 15 ml polypropylene tubes labeled 'Heavy Fraction' and ready for the next phase.

V. Technique 2: Microwave Digestion

1. Dry sediment was mixed with 3 ml of hydrochloric acid (HCl), 5 ml of nitric acid (NNO_3) in 50 ml Teflon microwave pressure vessels.
2. Samples were then mixed with 2 ml of hydrogen peroxide (H_2O_2); this was added drop by drop to avoid over spilling.
3. Samples were sealed and placed in the microwave for 60 minutes at a pressure of 80 psi, followed by a 15 minute cool down.
4. Samples were removed from the microwave, opened under the fume hood, another 2 ml of H_2O_2 were added to the samples and mixed using a glass-stirring rod.
5. Samples were sealed and placed in the microwave for a second digestion with the same setting as in step 3 (80 psi for 60 minutes and a 15 minute cool down).

6. After the second digestion, samples were removed from the microwave and uncapped in the fume hood.
7. Microwave pressure vessels were swirled gently for 20-30 seconds (to mix contents) and all contents were transferred to 15 ml polypropylene tubes labeled 'Heavy Fraction'.
8. The 15 ml tubes containing sediment (and chemical residue) were centrifuged for 5 minutes at 3000 rpm to form a well-consolidated pellet and the supernatant was poured off.
9. Tubes were filled to the '14 ml' line with distilled water, vortexed, centrifuged for 5 minutes at 3000 rpm, and the supernatant was poured off.
10. Step 9 was repeated to remove any residual chemicals and the sample was then ready for the next phase.

VI. Phytolith Flotation

1. A solution of 2.3 g/ml of sodium polytungstate (Na_6 [$H_2W_{12}O_{40}$]) was prepared and drawn through a 1µ microfiber filter twice, using a vacuum pump.
2. Samples were mixed with 5 ml of sodium polytungstate in the 15 ml tube and vortexed until pellet was fully disaggregated.
3. The tubes were centrifuged for 3 minutes at 3000 rpm and then were gently moved from side to side so the supernatant of clean sediment was not adhering to the sides of the tube. Once the sediment particles were rinsed from the sides of the tubes they were centrifuged for 5 minutes at 3000 rpm.
4. The supernatant was poured into another set of 15 ml tubes labeled 'Light Fraction'.
5. Steps 2-3 were repeated three times until very little material was visibly floating above the sodium polytungstate.
Note: It is necessary to remove the sodium polytungstate from the 'Light Fraction' tube through dilution, centrifugation, and the pouring off of the supernatant (this is set aside for re-filtering and reuse). The frequency with which this needs to be done will depend on the amount of phytolith extract that accumulates at the bottom of the 'Light Fraction' tub
6. After the fourth flotation both the 'Light Fraction' and 'Heavy Fraction' tubes were filled up to the '14 ml' line with distilled water to remove the remaining sodium polytungstate through centrifugation (this was also set aside for re-filtering and reuse).
7. Material in the 'Heavy fraction' tube was transferred back its original 50 ml tube for storage.

VII. Second Clay Removal

1. After the flotation step is completed distilled water is added to each tube up to the '14 ml' line, vortexed until the pellet is fully disaggregated, and centrifuged for 75 seconds at 1000 rpm.
2. The supernatant was removed using a 5 ml syringe with a 5 cm long needle, leaving at least 1 cm of the supernatant in the tube.
3. Steps 1-2 were repeated until the supernatant was transparent.

VIII. Extract Drying and Mounting

1. Remaining extract was dried in an oven at 70° for 14 hours.
2. About 5 mg. of the phytolith extract were mounted in Cargille "Type B" Immersion oil.

Results:

The two sets of slides from the two extraction techniques were compared under an Olympus BX51 microscope. It was found that samples prepared using the hot water bath method (V. Technique 1) for digestion produced slides with a greater number of humic colloids (making visibility of phytoliths difficult) than the exact same sample from the microwave digestion method (V. Technique 2). This may be due to the fact that these are highly organic soils and that in the hot water bath technique the organic digestion (HNO_3 and H_20_2 addition) occurs with wet rather than dry sediment and each addition of H_2O_2 is more diluted causing chemical waste (18 ml/sample used with hot water bath as opposed to 4 ml/sample used with microwave extraction). It would be interesting to repeat the procedure by drying sediments in between carbonate and organic removal however, this would make an already lengthy technique even more time consuming.

The microwave digestion technique has the advantages of producing cleaner results, faster processing, and less chemical waste than the hot water bath technique however, it presents a higher cost investment than using a hot water bath to digest samples. The microwave-digested samples produced much cleaner samples under the microscope, with clear visibility differences of phytoliths between both methods. These differences in visibility of phytoliths in these samples could eventually affect the results produced by each method, making the consistent use of one procedure advisable for all samples that are going to be studied together.

Works Cited:

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