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Preparation Of Soil Samples For Light And Transmission Electron Microsopy

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Introduction:

Soil structure influences water supply to plant roots, aeration, water infiltration rates, suitability of soil medium for seed germination and growth, growth of plant roots, drainage, evaporation, mechanical strength, and workability (Dexter 1988). Adequate description of soil structure for cultivation, engineering, or remediation is typically done by light microscopy and transmission electron microscopy. Literature exists in numerous sources for preparation of soils for microscopy, but often preparation steps are left out due to the shortening of Methods Sections in journal articles to conserve print space. I present here, protocols I've used for preparation of tropical soils (Oxisols) for microscopy.

Light microscopy:

The best reference for the preparation and analysis of soils by light microscopy is "Soil Microscopy and Micromorphology" by E.A. FitzPatrick, 1993. To prepare soils for examination by petrographic microscopy, approximately 30 mL of soil is transferred gently, by plastic spatula, to 50 mL Nalgene polypropylene beakers. The soils are first air-dried at room temperature (20°C) to constant mass. This usually takes 3-4 days, depending on humidity, and during this time, the soils are covered loosely with a paper towel or a filter. After air drying, the soil is dried in an oven at 40°C for 48 hours. The soil is then placed into a vacuum dessicator and mechanically evacuated to -1 atmosphere over a period of ten minutes. Under vacuum, LR White Acrylic Resin is poured slowly, to reduce bubble formation, into the soil. The soil and resin are further evacuated for another 10 minutes, removed from the vacuum, covered with aluminum foil, and left at 60°C to harden for 24 hours.

The hardened resin-soil block is cut with a diamond saw and mounted onto a glass slide with UV Cement for final trimming and polishing. Reduction of sample thickness to 30 μm is accomplished by polishing on a rotating lapping plate, first with silicon carbide and finally with alumina grit. The thin section is completed by mounting a coverslip with UV Cement.

Thin sections are typically observed with a polarizing light microscope, this allows birefringence measurements and interference figures to identify minerals in the prepared sections. An example micrograph is shown in figure 1.

Transmission Electron Microscopy:

A great amount of care is taken to preserve the soil structure via the preparation procedures that follow. However, in the TEM, the electron beam amorphises the specimens, as can be observed directly in the electron diffraction pattern, where a distinct electron diffraction pattern, indicating the presence of crystalline minerals, disappears after 30 to 120 seconds (Sudo and Takahashi 1971). To prevent the amorphizing of crystalline material, diffraction patterns should be collected by setting up for diffraction on a nearby area and then translating to the area of interest after the instrument is adjusted. Photomicrographs should be taken in a similar fashion, with focusing performed on a nearby area to limit damage to the feature of interest.

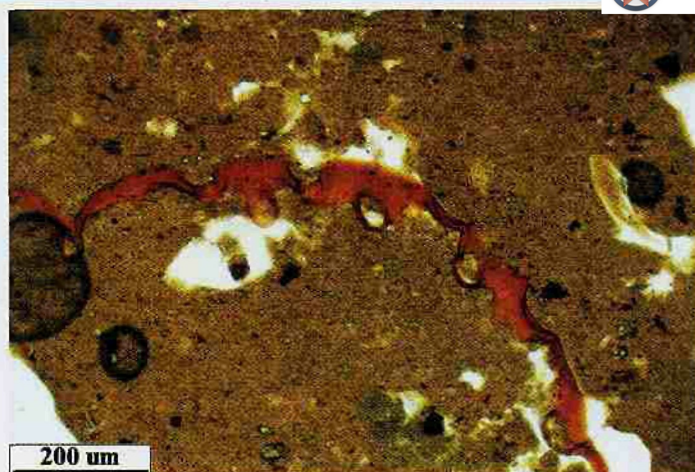


Figure 1. Very humified organic material cutting across the soil matrix. It has a striking reddish brown color, and is isotropic under crossed polarizing filters. The speckling of the matrix is due to secondary crystalline iron oxides.

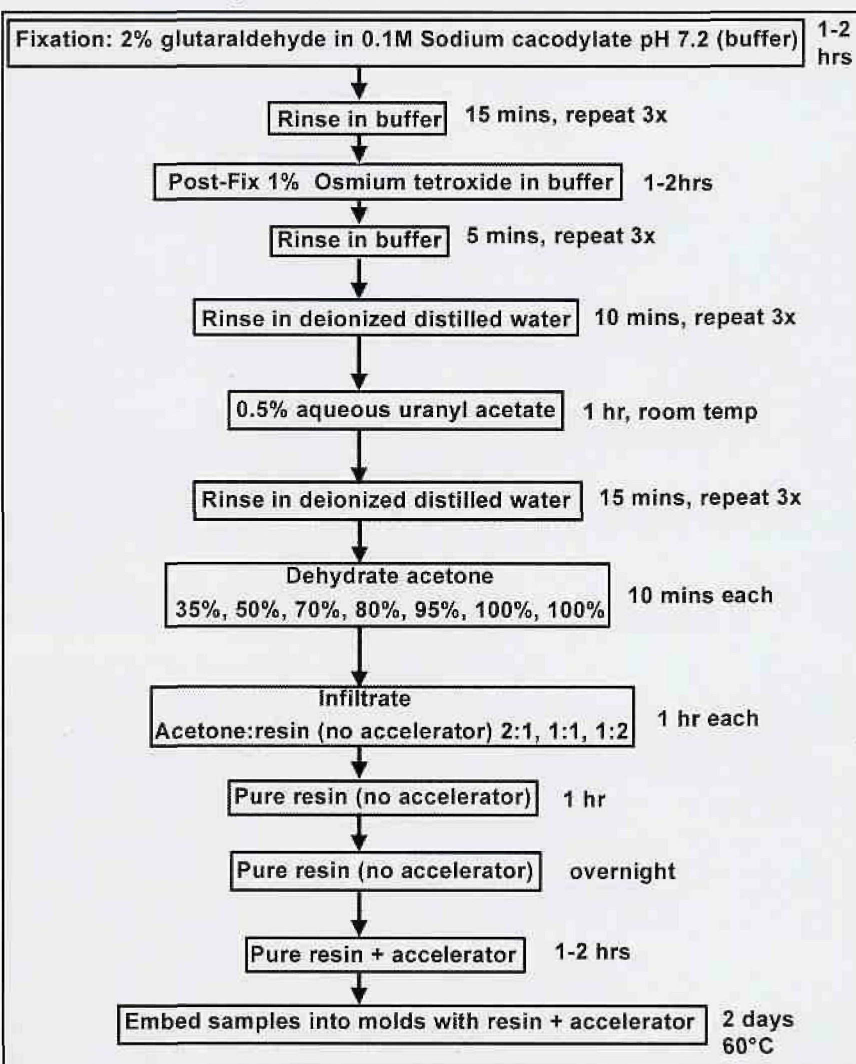
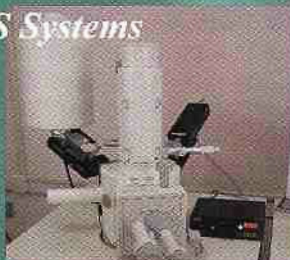


Figure 2: Flow chart outlining general procedures used to prepare soils for analysis by TEM.

Piecing Together Analytical Solutions



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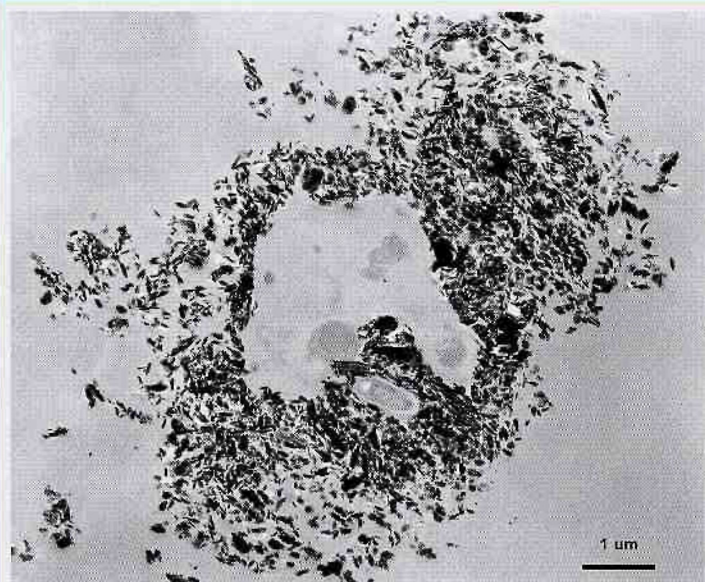

Figure 3: This aggregate has a core of organic material, as evidenced by the bacteria and bacterial polysaccharides. Clay minerals and iron oxides surround the aggregate.

Soil aggregates should be selected from different size ranges, to represent the structure of the soil, and also to have manageable sizes for imaging in the TEM. A stereomicroscope is very useful to screen out large primary mineral particles that don't represent the bulk of aggregates in the soil. The samples are usually selected by gently shaking sieves of appropriate mesh size with the soil and collecting the resulting aggregates. Typically I use "100-250 µm" and "< 53 µm" diameter sieves to select conveniently sized aggregates. I transfer the selected aggregates from the soils using moistened tweezers to lift (by adhesion between the water on the tweezers and the aggregates) into a warm 2% agar solution under a stereoscopic microscope. The agar with the aggregates are refrigerated overnight to gel. To select aggregates with sizes <20 µm and <2 µm in diameter in one operation, 20 g of soil is suspended in 2 mM NaCl solution (pH 8) and settled in 24 cm tall graduated cylinders. A settling time of 11 minutes in suspension yields aggregates in the sediment with diameters less than 20 µm. Aggregates with diameters of 2-20 µm can be found in the sediment obtained by settling the above soil suspension for 120 minutes in an evaporation dish 2.5 cm tall. Supernatant water is drawn off gently above the sediment with a capillary pipette. A warm (≈50°C) 2% agar solution is added to the sediment and solidified in a refrigerator overnight. The removed supernatant water solution (containing the < 2 µm fraction) is studied directly in the transmission electron microscope by evaporation onto formvar and carbon coated TEM grids.

After solidification in agar, the samples are trimmed to approximately 1-3mm sized cubes for processing. The steps used in the chemical processing are outlined schematically in figure 2.

The soils are fixed in a 2% glutaraldehyde solution with a 0.1.M sodium cacodylate buffer at pH 7.2 (hereafter called buffer) for 1-2 hours with gentle rocking agitation. The soils are then rinsed three times for fifteen minutes in buffer. Post fixation staining is done with 1% OsO_4 in buffer for 1-2 hours. Samples are rinsed in buffer three times for 5 minutes with agitation, and then rinsed three times for 10 minutes in distilled water. The samples are stained overnight at 4°C in the dark with 0.5% aqueous uranyl acetate and then rinsed 15 minutes three times with distilled water. Samples are dehydrated by rinsing for 10 minutes with increasing concentrations of acetone: water mixtures (35%, 50%, 70%, 80%, 95%, 100% and 100%). After dehydration, the samples are infiltrated with Spurr's epoxy resin (Spurr 1969) and acetone mixtures. Acetone:resin mixtures of 2:1, 1:1, and 1:2 are rinsed with the samples successively for 1 hour each. The samples are then rinsed with pure resin for 1 hour, and again overnight. Accelerator is added to the resin and the samples rinsed with resin plus accelerator for 1-2 hours. Finally, the samples are placed into beam capsule molds with accelerator and resin and left for 2 days at 60°C to harden.

An alternate method for preparing aggregates utilizing Nanoplast water-soluble resin can also be followed. Samples are placed into a 2% agar solution, solidified, and then prepared following the technique of Weyda (1990), instead of the chemical supplier's instructions. The samples were treated as above, but staining with OsO_4 is omitted as it interferes with polymerization.

After curing, the samples are trimmed and ultrathin sections (≈ 60nm) are cut on an ultramicrotome utilizing glass or diamond knives with a cutting speed of 0.1mm/sec. Slow cutting speed is important as soils often contain hard materials in a soft matrix. Sections are deposited onto copper or nylon TEM grids coated with a support film of formvar and carbon for insertion into the transmission electron microscope. I prefer coated grids that are made in our laboratory because, those ordered from suppliers often contain contaminants.

Some sections can be stained a second time to highlight organic features. The grids with sections are rinsed for 10 minutes in 2% aqueous uranyl acetate and then with five distilled water rinses. The grids are then stained with a lead citrate solution for 5 minutes and rinsed five times in distilled water.

Organic materials are seen as a slightly darker background shade in the TEM, compared to the embedding resin, due to staining by OsO_4 and uranyl acetate. Elemental mapping by EDX spectroscopy shows that these regions selectively contain osmium and uranium. Thus, the chemical staining technique used is very specific for organic material and did not significantly stain minerals or the embedding resin. Secondary staining of soil aggregates with well preserved organic structures serves to highlight some detail in the organic matter, particularly of cell walls. Gold shadowing of grids from the <2µm fraction can also be done to highlight fine humic organic materials. A 10 nm coating of gold is evaporated with a Vacuum evaporator (DV-502A) using a tilt angle of approximately 15° onto the grids. Figure 3 shows a typical result in the TEM from a < 53um diameter aggregate. Processing of soils for TEM is difficult, and often the chemical methods used have to be altered because of interfering components such as swelling clays. Feel free to contact me at my email address and I'll try to help if I can. ■

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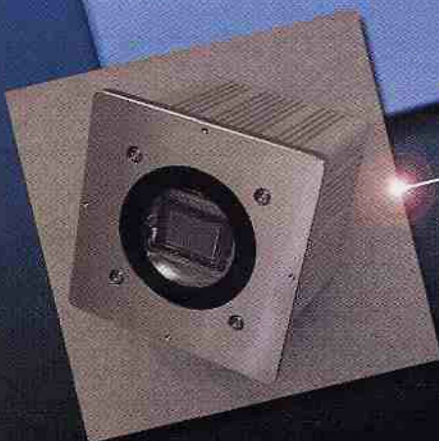

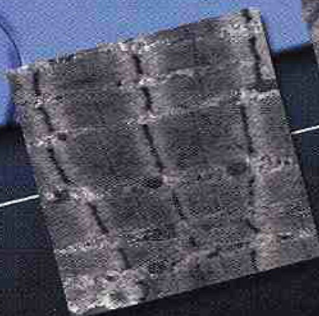

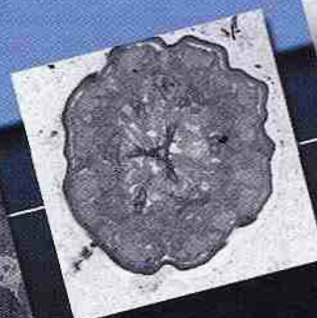

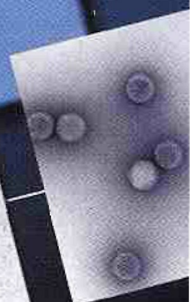

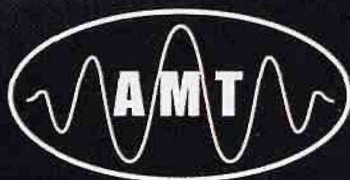

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