Botany 330

Sample Preparation Methods and Literature for Diatoms

Bob Mitchell, 1980

Cleaning

A. Hydrogen Peroxide

1. Add 75ml 30% H_2O_2 to sample in 1000 ml beaker.
2. Let stand for 24 hours. (Avoid evaporation.)
3. Add pinch of $K_2Cr_2O_7$ (potassium dichromate).
4. Let stand for 2 hours, then boil.
5. Add distilled water, let settle, decant. Repeat until liquid is colorless.

B. Potassium Permanganate

1. Add HCl to sample until no further reaction.
2. Add distilled water, let settle, decant.
3. Add 20ml H_2SO_4 and 10-15ml saturated $KMnO_4$ solution until sample turns purple.
4. Add 10ml oxalic acid until solution is colorless.
5. Add distilled water, let settle, decant. Repeat (8-10x).
6. Add 1-2ml strong ammonia to final washing to prevent clumping of cells. Wash again. (I have not tried this but it sounds a bit questionable since the frustules will dissolve in a base.)

C. Nitric Acid (other acids form precipitates with hard water)

1. Put sample and an equal volume of conc. HNO_3 in a Kjeldhal flask (or 600ml beaker). Add boiling chips.
2. Boil for about 20-30 minutes or until reaction ceases.
3. Add pinch of potassium dichromate until no effervescence. (This step seems only to be useful when you are working with a lot of organic matter e.g., sediment or epiphytes on their host).
4. Add distilled water to sample in a beaker, let settle (at least 4 hours), decant. Repeat until pH = 7 with litmus paper.

D. Plankton (lightly silicified forms)

1. Preserve and let settle, decant.
2. Add 95% EtOH or Chlorox bleach, let sit for ± 24 hours.
3. Decant, add distilled water, repeat.

Slide Preparation

1. Wipe cover slips (#1.5) with a very dilute solution of Photo-flo or alcohol to remove residues from glass. [Don't add Photo-flo to diatom suspension because it will dissolve the frustules!]

2. Dilute suspension to convenient density. If suspension is dilute spread directly on cover slip with a drop of dilute Photo-flo. If suspension is concentrated fill cover slip with Photo-flo solution and then add a few drops of the diatom suspension.

3. Let dry at room temperature overnight. A cover helps keep out dust.

4. In a fume-hood place a microscope slide on a hotplate and add ± 5 drops of mounting resin (hotplate should be set just lower than temperature where resin will boil).

5. Quickly invert cover slip onto resin and let toluene boil off until bubbling slows.

6. Remove microscope slide from hotplate and press out bubbles and excess resin. Let cool.

7. Trim excess resin from edges with razor blade.

8. This may be altered by adding resin to cover slips with a dried diatom suspension and letting the solvent evaporate overnight at room temperature, then inverting them onto the hot microscope slides. This may help with more complete embedding.

Note: The acids, oxidizers, solvents, and resins used for these methods are nasty! Some are known carcinogens and the others are probably as bad. Use a hood or very well ventilated area.

Literature

A. Methods

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