B.Sc 2rd Year [IVth Semester]: Paper 403 Practical Work on "*Pollen techniques & Palynology*"

Click the Link:
https://www.microscopemaster.com/pollen-under-the-microscope.html

Modern Pollen Preparation Protocol (V. 1-1)

Pollen reference slides made from specimens collected in the field or acquired through a botanic garden are a valuable aid in the identification of fossil pollen, and are especially important when working in a region for which the flora is poorly represented in an existing reference collection. This protocol details how to make slides of modern pollen from flowering plants for reference use in pollen analysis.

A. Preparing the specimens

Equipment:
Tweezers, razor
blade, seeker Smallglassvialswithlids Low-power stereo microscope

1. Identification

Pollen can be recovered from fresh flowers, or from pressed or dried specimens. For reference material to be useful to others, accurate identification of the plant is essential. If you have material for which you are unsure of the identification, for example from a plant growing abundantly near the location of a core, attempt to identify the plant through printed guides or the help of a botanist. Consult a reference work, such as Flora Europea, and confirm the precise identity of your specimens.

2. Isolation of anthers

The aim in the early stages of the preparation is the isolation and concentration of the pollen-bearing flower parts (anthers). Examine your specimen under magnification and using tweezers or a razor blade, cut or pull apart the outer parts of the flower to expose the pollen-bearing anthers. These can then be collected and sealed in a labelled vial. This stage allows you to confirm that there is pollen present. Flowers which are on the point of opening usually provide more pollen than those which are already open; flowers which are fully opened may have already shed their pollen. Anthers prior to dehiscence (shedding their pollen) can usually be easily distinguished by their golden colour and the presence of freely-falling pollen; anthers which have already dehisced are usually darker brown. Of course, in the case of very fragile or small flowers, it may be preferable to collect entire flowers. *Photo: collecting anthers of Cistus ladanifer.*

This preparatory stage also provides an idea of how many anthers should be collected. Different plants produce very different amounts of pollen, and no set number can be prescribed as sufficient. Usually, inspection under magnification provides an indication as to the abundance of the pollen present. While the anthers of a single, large and polleniferous flower may be quite sufficient, in the case of small or compound flowers, ten or twenty flowers may need to be included. *Photo: anthers from several different flower types ready for the next stages.*

3. Avoiding contamination

If you are preparing several specimens, be careful of cross-contamination. Working on a stack of scrap paper allows you to start a new sheet for each new specimen. Rinse and dry your implements and hands between specimens, and periodically wipe down your work surface with damp paper towels. If you are preparing a group of specimens from a closely related group of plants, it is even more critical to avoid cross-contamination. Metal implements can be flamed over a Bunsen-burner between specimens.

B. Laboratory procedure for pollen preparation

These stages represent a reduced version of the method used for recovering pollen from sediments (compare *Pollen Preparation Protocol*). In summary, the stages required here are:

1.Hot 10% NaOH
2.Sieve
3.Water washes
4.Acetolysis.
5.NaOH. 1% and safranin stain (optional)
6.Dehydration in TBA
7.Recovery of residue and suspension in silicone oil

If unfamiliar with the Pollen Preparation Protocol, the detailed explanations for the equivalent stages should be consulted. Here, the important additions and variations at each stage for modern reference samples will be outlined.

1.Hot 10% NaOH

Empty the contents of the specimen vials into large centrifuge tubes. Cover the plant material with NaOH (tubes approx. 1/4 – 1/3 full). Using ball-ended glass rods, assist the maceration of each sample by driving out air with a crushing or grinding action. Place in water bath at 90° C for 4 minutes.

2.Sieve

Set up a second set of centrifuge tubes with funnels and flamed sieves as for the standard protocol, except using small tubes rather than large ones. Empty the contents of the large tubes into the sieves. Wash the contents of the sieve with a gentle stream of DI water from a wash-bottle. Unlike the standard procedure, it is not necessary to blast the contents of the sieve, attempting to wash through all the fine material. The separation of the pollen from the flower parts in the samples will have been accomplished in Stage 1; too much washing in the sieves will only force through unwanted organic material.

Balance tubes in centrifuge holders. Centrifuge, 5 minutes at 3500 rpm. (The longer and faster settings recommended in this protocol reflects the small size of the residues)

Decant supernatant. It is possible that virtually no residue will be visible from this point onwards. Do not despair or discard the tube?you may easily still have sufficient pollen to work with at the end.

3.Water washes

Whirlimix. Top up with DI water. Balance. Centrifuge. Decant.

Repeat until the supernatant is clear and colourfree; 3 washes are usually sufficient.

4.Acetolysis

Whirlimix. Top up with glacial acetic acid, CH3COOH. Balance. Centrifuge. Decant.

Prepare acetolysis mixture (9 parts acetic anhydride to 1 part concentrated sulphuric acid) following same method and precautions as standard protocol, allowing 4-5ml per sample. Pour mixture into tubes and place in water bath at 90° C for 4 minutes.

Remove from water bath. Top up with glacial acetic acid. Balance. Centrifuge. Decant down fume-cupboard sink.

Whirlimix. Top up with DI water. Balance. Centrifuge. Decant.

5.NaOH. 1% and safranin stain (optional)

(If staining is not desired, this stage may be left out; move to Stage 6.)

Whirlimix. Add a squirt of 10% NaOH. Whirlimix. Top up with DI water. Balance. Centrifuge. Decant.

Whirlimix. Add one drop of 0.2% aqueous safranin solution. Whirlimix. Top up with DI water. Balance. Centrifuge. Decant.

6. Dehydration in TBA

Prepare TBA as per standard protocol, following the same precautions. Fill tubes to half full. Balance. Centrifuge. Decant into beaker containing water; pour down fume cupboard sink.

7. Recovery of residue and suspension in silicone oil.

Whirlimix. The residue in the tubes will be transferred to labelled glass vials by using glass Pasteur pipettes with a rubber teat. This method is useful for samples where very small amounts of residue are present. Draw up the residue from the first tube into a new pipette and release into a labelled vial. Add another squirt of TBA to the tube, whirlimix, and transfer again into the vial. Repeat with additional squirts of TBA until all traces of residue have been transferred into the vial. Repeat the procedure for each subsequent sample.

Place the vials in the centifuge holders. Balance (water in empty centrifuge holder slots). Centrifuge. Decant TBA into waste beaker containing water; dispose in fume cupboard sink.

Add a few drops of silicone oil to the vials, and stir with cocktail stick to suspend the residue. Be careful not to dilute the residue too much by adding a lot of silicone oil.

Add only a few drops; additional oil can be added as the TBA dries over the subsequent 24-48 hours, and again when making the slides.

C. Making permanent slides of reference pollen residue

Equipment:

PolleresiduesfromPart B
Cocktail sticks
Glass slides and circular cover-slips (13-16mm diameter)
Small crucible
Paraffin wax
Hotplate
Glass pipettes and rubber teats
Gentle heat source (Meths burner)
Stand for holding crucible over heat
Upturned metal tray for cooling slides
Razor blades, Meths, and paper towels

WARNING! Use caution in handling hot wax and near the heat source and hotplate.

1. Heat wax in crucible over the burner until liquid. *Photo*
2. Set hotplate to highest setting. Place slides and cover-slips on hotplate to warm. *Photo*
3. Have the upturned tray ready to receive slides. *Photo*
4. Have pipette with teat and cover-slips within easy reach.
5. Have residue ready to apply with cocktail stick.
6. In relatively quick succession:
   a. Add one drop of residue to a hot slide, with slide still resting on the hotplate.
   b. Place a cover-slip squarely over the residue drop
   c. Draw a small amount of very hot wax into pipette, and release onto the slide at the edge of the coverslip.
   d. Allow the wax to run under the entire cover-slip, surrounding the residue.
   e. Pick up the slide and place onto cooling tray
7. Use a razor blade to pare away unwanted wax from the slide.
8. Use paper towels and Meths to polish away any waxy residues on the slide or coverslip. *Photo*
9. Label the slide.

It is advisable to take a look a the first slide made from a new residue to check the concentration of pollen. Ideally, there will be many grains in a single drop, but not so many that the grains touch or are crowded on the slide. If the residue is too concentrated, dilute with additional drops of silicone oil and stir to mix.
Making the slides takes practice, so be prepared to lose some slides in the learning process. Problems most often arise in the placement of the wax. If the wax does not run smoothly under the cover-slip, or if it starts to solidify before encircling the residue, either the slide, cover-slip or wax may not be warm enough. The wax flows most easily when very hot; however, watch for signs of smoking!

Ideally, no air bubbles will be present on a reference slide. When the residues are stirred, small bubbles are incorporated which take some minutes to escape, so do not stir the sample immediately prior to lifting a drop onto a slide.

With practice, slides can be made quite quickly, even working through stage 6 in parallel on two or three slides at once. It is more efficient to work first through stages 1-6 on several slides, using a temporary label in permanent marker to distinguish between slides of different residues. Then progress to stages 7-9. Trim excess wax from all the slides with a razor blade, then take the entire batch on a tray into the fume cupboard where a paper towel soaked in Meths can be used. Meths should be used in a fume cupboard and latex or vinyl gloves should be worn. In fact, the time needed to make the slides (stages 1-6) is short compared with the time needed to clean up and label the slides. This means that you can afford to be choosy about keeping only the best slides.

Compiled by William Fletcher

WASHING UP PROTOCOL

All equipment that has come into contact with pollen, except the sieves should be washed in this way.

1) Wash and scrub vigorously in normal detergent and warm water, then placed under running water.

2) Transfer to a bowl containing deionised water and 50-100ml of Decon 90 (Alkaline and will burn skin). Soak for 12-48 hours.

3) Rinse in deionised water and fully immerse in a 1% HCl solution (cover bottom of bowl with 7% HCl and half fill with deionised water.

4) Stack in drying basket and place in ~50°C in glassware drying oven. Put your tubes away!!!!

LABORATORY SAFETY

PROTECTION

- ALWAYS use the protection provided.
- ALWAYS wash your gloves and other impervious clothing before you remove them.
- ALWAYS wash your hands before you leave the work area.

SPILLAGES

- The contaminated area must be hosed with plenty of water.
- Spillages on clothing - neutralise with sodium bicarbonate solution.

FIRST AID

1) Skin

- Remove contaminated clothing (NB protect your hands with gloves).
- Flood skin with clean cool water to remove acid. Send to Accident and Emergency Unit without delay, continuing the following during transit.

2) Eyes

- Irrigate with clean cool water for at least 20 minutes. This can be continued during transit to hospital.
- Send to Accident and Emergency Unit or local eye hospital.
- In all cases inform hospital of the cause of the injury.
- Report splashes on skin and eyes to supervisor/employer.

FIRST-AID TRAINING: It is the responsibility of your employer to ensure that there is an adequate number of employees on site trained in appropriate first-aid procedures.
Training should be given by organisations approved by HSE. If you have an occupational health department, the doctor or nurse will be able to advise you on how to carry out the different procedures in your workplace.

LEGAL REQUIREMENTS: The Control of Substances Hazardous to Health (COSHH) Regulations 1988 apply to hydrofluoric acid - see Approved Code of Practice COP29, *Control of substances hazardous to health*, available from HMSO (ISBN 0 11 885468 2).

The Health and Safety (First Aid) Regulations 1981 apply to all aspects of first aid at work - see Approved Code of Practice COP42 *First aid at work*, available from HMSO (ISBN 0 11 885536 0).