POLLEN EXTRACTION PROCEDURE

FIRST DAY OPERATIONS (13/07/ 2021)

1. Sample weighing;
2. Insertion of samples into test tubes;
3. Pouring soda NaOH 10% into test tubes to break down organic component, all is then mixed with stirring rods;
4. Boiled the test tubes in a water bath for just over 10 minutes. In the meantime, a Becker filled with distilled water was also heated;
5. Centrifugation of the material for 2 minutes at 3000 rpm (revolutions per minute);
6. Decantation of the supernatant (pouring off the liquid part of the tube);
7. Carried out 4 washes with previously heated distilled water. That is, insert the water into the test tube, centrifuge for 2 minutes at 3000 rpm and decant, all repeated four times. Four times instead of the normal two because the samples were still not clean enough after two;
8. Under fume hood, insertion of a few drops of HCl into the test tubes to remove any limescale, only sample no. 10 showed a little effervescence reaction;
9. This was followed by bringing all the test tubes up to level by adding 10 c.c. of distilled water. Leaving the test tubes to stand under a hood.

SECOND DAY OPERATIONS (23/07/2021)

1. On day 22/07/2021, acetolysis was carried out utilizing the Erdtman 1960 procedure [1]: before carrying out acetolysis, we proceeded to dehydrate the samples with glacial acetic acid for 20 minutes, with centrifugation and decanting of the supernatant.
2. Before starting work on the samples, we turned on the fume hood, waiting about ten minutes to pass;
3. We washed the test tubes with a couple of centrifugations of just over 5 minutes at 3000 rpm with distilled water;
4. Then the material was transferred from the test tubes to the Eppendorf tubes using the following procedure: inserting 0.5 c.c. of ethanol into the test tubes, swirling them, pouring the contents into the Eppendorf tubes, centrifuging the tubes for 3 minutes at

2500 rpm, decanting them as to leave inside only the sediment of the sample. In order to ensure the transfer of as much material as possible from the tubes to the Eppendorf tubes, the procedure was repeated several times;

5. Addition of a drop of glycerine for Eppendorf tubes;
6. Drying the Eppendorf tubes in the oven for about 1 hour at 60 °C;
7. Preparation of the slides.

REFERENCE

1. Erdtman G., The acetolysis method, a revised description, in *Svensk Bot. Tidskr*. 1960, 54, 4, pp. 561-564.