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## Protocol for pollen and dinocyst analysis in marine sediments

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**We use this protocol and it's working**

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## Abstract

The protocol is dedicated to the preparation of sediment samples from marine cores to analyse pollen and dinocysts.

## Materials

### Devices and lab equipment

Fume hoods  
Centrifuge  
Hot plate  
Ultrasound device (model Fisher sonic dismembrator with probe)  
Ultrasonic tank  
Stove  
Orbital shaker  
Balance  
Vortex mixer (tube shaker)  
Diaphragm pump  
Milliq water system

### Lab material

100 ml round-bottom tubes  
50 ml conical bottom tubes  
50 ml tube rack  
100ml tube rack  
1L beakers  
Ceramic evaporating dishes  
1000 ml pissettes  
Pipette pear  
Pasteur pipettes  
Glass rods  
150  $\mu\text{m}$  mesh sieves  
10 or 5  $\mu\text{m}$  nylon mesh screens  
2.5 cc eppendorf tubes  
Slides  
Coverslips

### Chemicals- products

HCl  
HF 48%  
HF 70%  
Histolac  
Glycerol 99%  
Lycopode tablets  
Fuchsin (only for dynocyst analysis)  
Gelatin leafs (only for dynocyst analysis)

## **Protective equipment**

Gloves resistant to chemicals

Laboratory coat

Safety footwear

Apron resistant to chemicals

Visor resistant to chemicals

Lab goggles

HF bottle-top dispenser

HCl bottle-top dispenser

Nitrile gloves

Full face mask for respiratory protection with adequate cartridge in the event of an incident

## **Troubleshooting**



## **Sediment preparation and volume and weight estimation**

### **1 Sampling/ Subsampling**

1.1 Take a subsample between 2 and 5 g of sediment (or 3 to 5 mL).

### **2 Volume and weight estimation**

2.1 The sediment is dried in an oven at 40°C for one night or more, depending on the water content.

2.2 In a 25 ml measuring cylinder, put 15 ml of distilled water.

2.3 Place it on a balance and tare it.

2.4 Add the sediment.

2.5 The water column increases, record the weight and the volume.

2.6 Let soak for one or two hours (depending on the nature of sediments) for helping disaggregation.

## **Preparation of the sediment samples before acid attacks**

### **3 Wet-wash sieving step.**

3.1 Take a 150 µm mesh sieve with a diameter of 10 cm, place it on a 1 L beaker.

3.2 Empty the contents of the cylinder onto the sieve, and wash gently with tap water until is clean.

When washing, be careful not to overflow the water of the beaker.

- 3.3 Rinse the residue > 150  $\mu\text{m}$  with distilled water and recover it in a dish. This fraction will be dried in oven for the study of other proxies such as foraminifera.



Photo 1

- 3.4 The lower fraction recovered in the beaker is decanted for at least 48 hours. Do not forget to cover the beakers to avoid any contamination with modern pollen.

4 **Separation of the fine fraction containing the palynomorphs.**



Photo 2

- 4.1 Suck out the water with a lab diaphragm pump equipped with a hose having a the end a nozzle forming a 90° angle (Photo 3). This nozzle prevents any accidental aspiration of the decanted residue at the bottom of the beaker.



Photo 3

- 4.2 Collect the residue in a 100 ml round-bottomed polypropylene tube (cf. Photo 2).
- 4.3 Centrifuge at  for 7 minutes.
- 4.4 Eliminate the water.

## Acid attacks

### 5 **Attack with hydrochloric acid (HCl) to eliminate all calcareous organisms and particles.**

This HCl attack is done in three steps. It is important to carry out this attack correctly to achieve complete elimination of carbonates.

#### Safety information

Be sure to comply with the health and safety regulations for the chemicals used in the lab.



Photo 4: HCl attack

5.1 Resuspend with a tube shaker (see Photo 5).



Photo 5 : Tube shaker / Vortex mixer

- 5.2 Put one or two Lycopodes pellets in the tube, the number of pellets being defined according to the supposed pollen or dinoflagellate concentration.

Note

Lycopods (exotic grains) allow to estimate the concentrations of palynomorphs. Be careful, note the amount of lycopods per tablet in the laboratory booklet.

- 5.3 A first attack at **10% HCl**, stir with a glass stirring rod, leave to act for a few minutes, if the sediment is rich in carbonates it is important to start with HCl at a low concentration in order to avoid effervescence and also to avoid overflowing the tubes. The amount of HCl used for this attack is at least equivalent to the amount of sediments, but depends of their nature.

- 5.4 Continue with **HCl at 25%**, stir with a glass stirring rod for a few minutes and allow to react.
  - 5.5 Finish with an attack at **50% HCl**. Stir with a glass stirring rod for a few minutes and allow to react. This last attack is essential for the continuation of the manipulation. Make sure that the reaction is complete by adding 50% HCl. When there is no more effervescence in the tube, the reaction is completed.
  - 5.6 Centrifuge the 100 ml tubes for 7 minutes at  2500 rpm , only when there is no more effervescence.
  - 5.7 Remove the supernatant by pouring it all at once into a chemical waste collection can without accoup.
  - 5.8 Shake the residue with the tube shaker (see Photo 5).
- 6 **Attack with hydrofluoric acid to remove silica and silicates (48% HF)**  
 Be careful to respect the concentration of the HF, because if the sediments are silica-riched this can lead to strong reaction, with the risk of losing sediment.

#### Safety information

HF use must comply strictly with health and safety regulations, as the HF acid presents high health risks. Read the safety data sheet carefully before handling. Use specific personal protective equipment (gloves, closed shoes, protective apron and visor).

- 6.1 Add about 20 ml of cold **48% HF**.



Photo 6

- 6.2 Close the tubes with the appropriate cap but do not close completely to let degasing.
- 6.3 Place the tubes on an orbital shaker (see Photo 7).

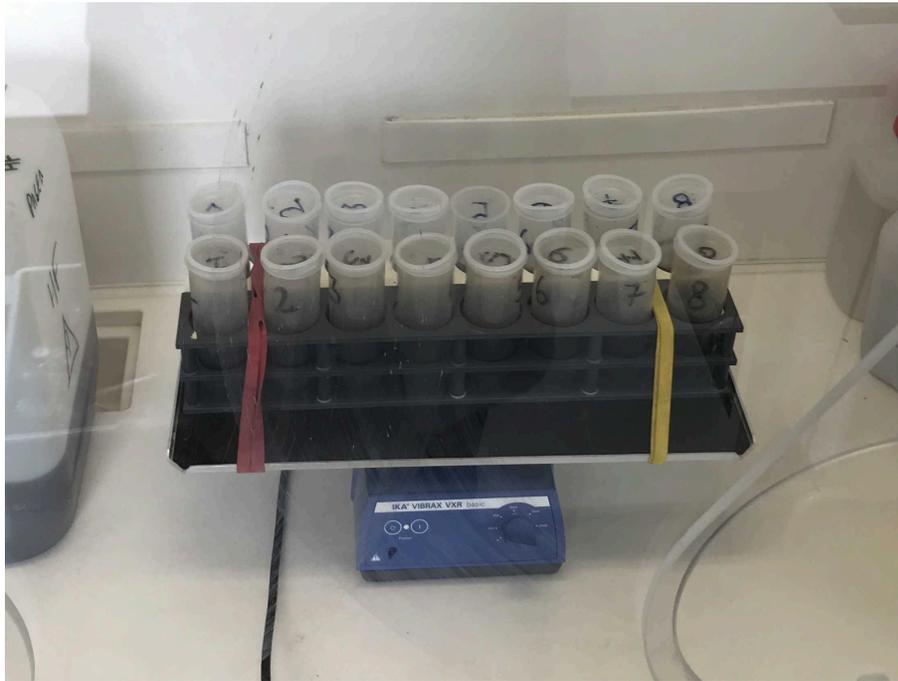


Photo 7: Tubes on the orbital shaker

- 6.4 Leave to shake for 10 to 12 hours minimum (overnight step).
- 6.5 Centrifuge the tubes again for 7 minutes at .
- 6.6 Remove the supernatant by pouring it all at once into a chemical waste collection can without accoup.
- 7 Second HF attack (70% HF)**
- 7.1 Resuspend with the tube shaker.
- 7.2 Add about **20 ml of cold 70% HF**.
- 7.3 Place the tubes on the orbital shaker and let shake for 10 to 12 h minimum (overnight step).

7.4 Centrifuge the tubes for 7 minutes at  2500 rpm .

7.5 Remove the supernatant by pouring it all at once into a chemical waste collection can without accoup.

## Rinsing with hydrochloric acid (HCl) to remove fluorosilicates

### 8 Rinsing with HCl step.

This HCl attack needs to be done before rinsing the samples with distilled water. Fluoride formation during the previous HF attacks can disrupt the rest of the manipulation; fluorides need to be removed.

#### Safety information

Be sure to comply with the health and safety regulations for the chemicals used in the lab.

8.1 Resuspend with the tube shaker.

8.2 Put about 25 ml of **cold 25% HCl**.

8.3 Put the tubes on the orbital shaker, let shake for 30 minutes minimum.

8.4 Centrifuge the tubes for 7 minutes at  2500 rpm .

8.5 Discard the supernatant by pouring it all at once into a chemical waste collection can without accoup.

## Rinsing with distilled water

### 9 Sample rinsing step with distilled water

9.1 Resuspend with the tube shaker.

9.2 Fill the tubes with distilled water.

9.3 Centrifuge the tubes for 7 minutes at  2500 rpm .

9.4 Eliminate the water, the residues are ready for filtration.

10 **Second rinsing step with distilled water** (  [go to step #9](#) )

## Filtration

### 11 **Sample filtration step**

We use a home-made filtration system (see photo n°4 for the description) but a commercially available filtration system can be used. This material is placed on a 2l vacuum flask connected to a vacuum tube (photo n°5). A 10 µm or 5 µm nylon mesh screen is used depending on the origin of the sediment (5 µm for sediment from the tropical regions and 10 µm for th other regions).

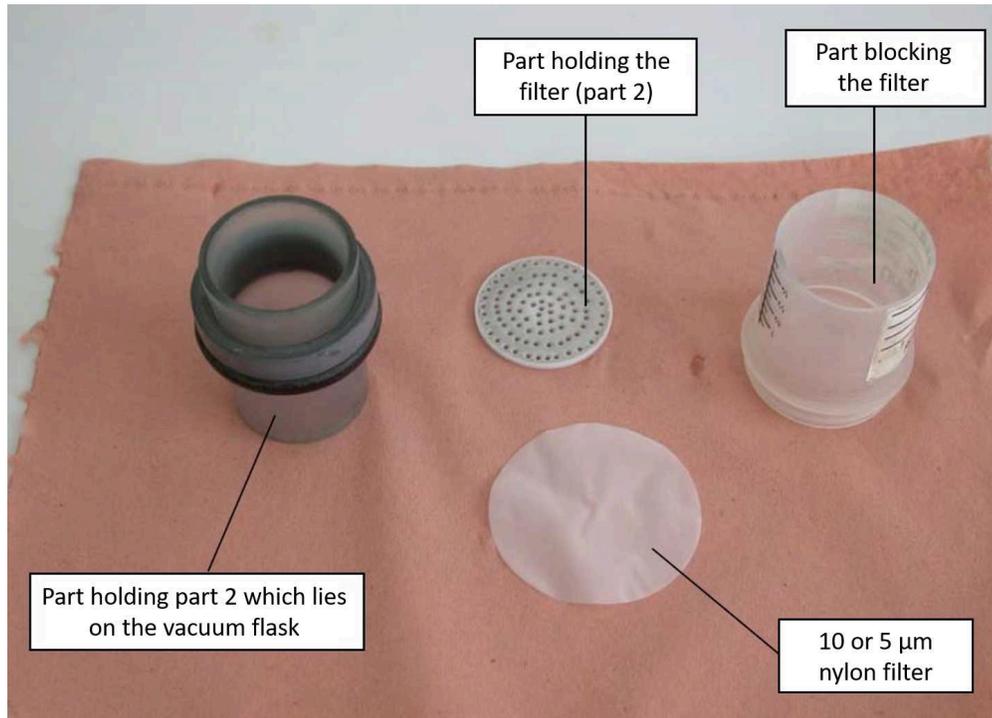


Photo 8: Filtration system



Photo 9: Filtration system mounting

- 11.1 Resuspend the pellet with the stirrer.
- 11.2 Pour part or all of the residue, depending on the concentration, on the 10  $\mu\text{m}$  or 5  $\mu\text{m}$  nylon filter depending on the origin of the sediments (see photo n°4).



Photo 10

- 11.3 Rinse with distilled water with a 50 ml wash bottle. The capacity of the washbowl is essential because it allows to have it well in hand and thus to press hard enough to have a powerful enough jet for the filtration.
- 11.4 When the filter clogs, apply ultrasound for 20 to 40 seconds (see photo n°5) and rinse at the same time with the pissette. The success of this operation is based on the coordination between the ultrasound and the sprayer. It is important to manage the time of the ultrasound (20 seconds, 2 times maximum), because the abuse can break the palynomorphs.  
Filtration is complete when the sample filtrate becomes clear.
- 12 **Transfer of the residue from the filter to an eppendorf tube**



- 12.1 Once filtered, collect the residue in a 50 ml conical tube, using the wash bottle. To recover everything, carefully remove the filter from the apparatus, and wash the filter by positioning it between your fingers using a jet of water to remove any palynomorphs that may remain on the filter.
- 12.2 Add glycerol 99% (extra pure) to the 50 ml tube. The amount of glycerol added should be ~1.5 times the amount of residue.
- 12.3 Centrifuge the tubes for 7 minutes at  2500 rpm , remove the supernatant with the vacuum tube equipped with the 90° nozzle in order to eliminate water from the residue.
- 12.4 Transfer the residue located in the 50 mL into a 2.5 cc eppendorf tube.
- 13 **Slide mounting**

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#### STEP CASE

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#### Slides for pollen analysis

- 13.1 Put a slide on the hot plate (T° 100°C).
- 13.2 Add a few drops of the mixture of residue and glycerine stored in the eppendorf tube. If the mixture appears too concentrated, add a drop of 99 % glycerol. Dose according to the desired concentration and homogenize.

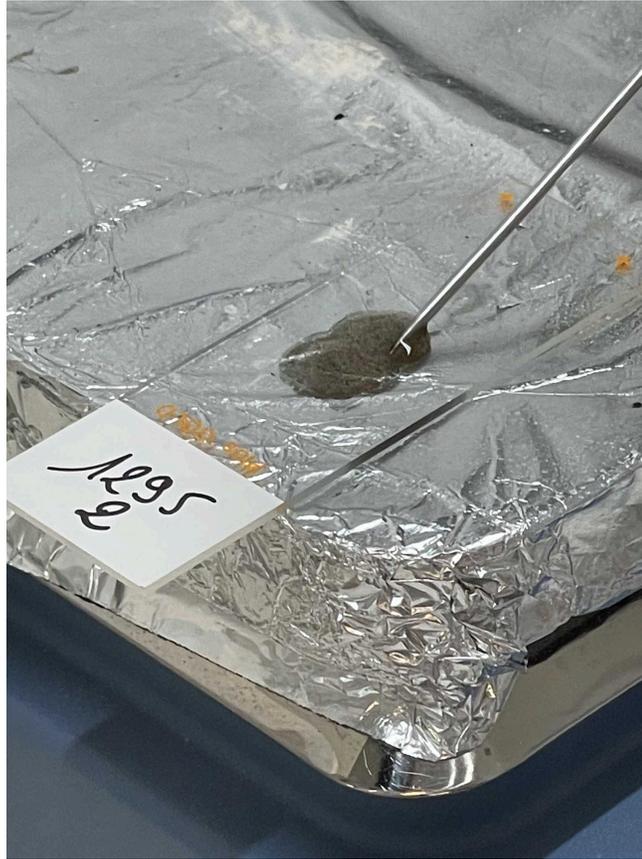


Photo 11: Slide mounting - steps 2

- 13.3 Let the eventual remaining water evaporate for a few minutes.  
During this time, prepare the coverslips by placing histolac on long edges.



Photo 12

- 13.4 Remove the slide from the hot plate.  
Put the coverslip on the slide.



Photo 13

13.5 Lute the two remaining sides (short edges) with histolac.



Photo 14: Slide luting

## Sample storage

- 14 For storage, a drop of thymol can be added to avoid the development of organism that may prevent pollen and dinocyst. Samples are stored in refridgerated room at 4°C .

## Microscopic analysis

- 15 Pollen, spores and dinocysts are counted using a light microscope. Palynomorphs are examined under the microscope at 400x magnification for routine observation and 1000x magnification for identification. Pollen and spore identification is carried out with the help of the EPOC laboratory's reference pollen collection and various pollen atlases.