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MICROPHYTOBENTHOS BIOMASS SAMPLING AND CHLOROPHYLL/ PHAEOPIGMENTS MEASUREMENTS

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by

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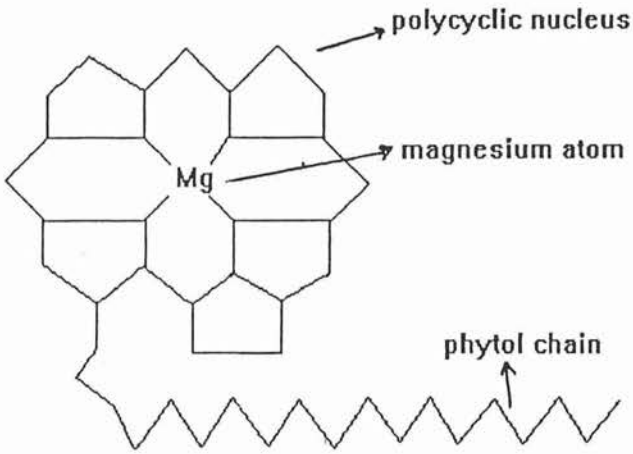
SAMPLING AND CHLOROPHYLL/PHAEOPIGMENTS MEASUREMENTS

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass. Microphytobenthos biomass assessment by a chemical method such as photosynthetic pigment extraction and quantitative chlorophyll determination gives good results and is more simple and rapid than methods based on cell counts.

The spectrophotometric method is one of the most widely used to provide an assessment of the main pigment, chlorophyll *a* and its pheopigments. The other chlorophylls (*b* and *c*) and breakdown products are only occasionally sought as taxonomic and physiological indicators. These compounds, if present, can interfere in the chlorophyll *a* determination. The overestimation of chlorophyll *a* due to pheopigments that absorb near the same wavelength can be corrected by acidifying the sample and remeasuring the absorbance at a nearby wavelength.



Chlorophyll structure

The chlorophyll *a* degradation on acidification can be simplified as follows:

chlorophyll *a* —(- phytol chain)→ chlorophyllid *a* —(- Mg)→ pheophorbid *a*

chlorophyll *a* —(- Mg)→ pheophytin *a* —(- phytol chain)→ pheophorbid *a*

The acidification results in magnesium atom and phytol chain elimination. The main breakdown products in marine waters are pheophorbid and pheophytin. Both are grouped together as pheopigments in this report.

1. SUMMARY

The sediments are first sampled by coring. If the core in cut in sections and a measurement made on each section, then the cores provide a vertical profile of chlorophyll *a b* and *c* concentration in the sediment . The sections are immersed in a solvent which extracts the pigments. Then, the absorption of the extract is measured on a spectrophotometer, both before and after acidification at different wavelengths.

2. SAMPLING METHOD

Samples of sediment are collected with plastic corers of 3 cm internal diameter and 20 cm long. Clear plastic corers are recommended in order to detect any disturbance of the core when sampling. The sediment surface has to be reasonably plane, without any irregularity. The corer is sunk into the sediment until 5 cm of the corer tops the surface of sediment. Then, the top of the corer is plugged with a rubber plug or a cap. The corer is gently removed from the sediment and another rubber plug or cap is put at the bottom of the corer. If the sampling is carried out by diving, it may be necessary to dig around the corer to extract it and to plug the bottom of the corer very quickly. The corer must be kept upright in a sample-holder.

Great care must be taken during the sampling and transport of the cores in order to prevent any disturbance of the sediment.

3. STORAGE

To make accurate measurements on chlorophyll, the extraction must be achieved as soon as possible (less than 4 hours after the sampling). If the extraction has to be delayed, the core may be frozen either as whole or in sections. If the transport to the laboratory takes a long time, the samples must be kept at a low temperature (ice box) and in darkness.

4. REAGENTS

4.1 Magnesium carbonate solution at 1%

1 g of magnesium carbonate is placed in suspension in 100 ml of water. This suspension must *be shaken immediately before use* to disperse the powder.

4.2 Solvent extraction

90 % acetone is commonly used as solvent for chlorophyll pigments. Acetone of analytical grade must be used. The 90% acetone must not be prepared in too large a quantity because of evaporation.

To prepare the solvent, 50 ml of distilled water is placed in a 500 ml volumetric flask with a pipette and made up with acetone until the mark.

4.3 0.3 mol.l^{-1} hydrochloric acid solution

A 0.3 mol.l^{-1} hydrochloric acid solution is prepared by adding 2.5 ml concentrated hydrochloric acid (sp.gr. 1.18) to 97.5 ml distilled water.

5. CORE SECTIONING

* If the corer contains water, drain the water from the sediment with a tube connected to a syringe.

* Remove the bottom cap and replace it with a piston.

* Remove the upper cap. Gently push up the core with the piston until the surface of the the core reaches the top of the corer.

* Put a 0.5 cm plastic ring on the top of the corer (for coarse sediments a 1.0 cm ring will suffice).

* Push the core cautiously until it fills the ring.

* Slide out the ring to excise the first sediment slice.

* Drop the sediment slice in a small glass or plastic jar of about 100 ml, equipped with an hermetic cap. Use only plastic containers compatible with acetone. It is preferable to use dark plastic containers that can be heated to 100 degrees centigrade.

A profile in the sediment can be obtained by repeating the above procedure on the length of the core. A 0.5 cm (or 1.0 cm) ring is usually used to excise the first 3 sections. 1.0 cm (or 2.0 cm) rings are used for the remainder. Put each slice in a different marked jar. The thickness of sediment needing sectioning depends upon the particular site being monitored.

6. PIGMENT EXTRACTION

Because the samples and extract are light sensitive, it is best to work in dim light and to put the samples in darkness as soon as possible.

* Add 15 ml 90% acetone to each jar containing a section of sediment.

* Add 0.5 ml 1% magnesium carbonate solution with a small syringe.

* Stir properly the sediment and the solvent.

* If the jar is transparent wrap in aluminium foil to avoid light exposure.

* Leave the extract for 20 hours in a fridge.

* After 20 hours, shake the samples and leave them to reach room temperature.

7. ABSORPTION MEASUREMENTS

7.1 Blanks determination

Two sorts of blanks are involved in chlorophyll spectrophotometric measurements: the cell blank and the turbidity blank.

To determine the cell blank (cb):

* Fill the two spectrophotometer 1 cm cells with 90% acetone.

* Auto-zero at 665 nm.

* Measure the absorption at 750 nm to give the blank cb_{750}.

* Then measure the absorption at 645 nm and 630 nm to give cb_{645} and cb_{630}.

The blank has to be checked every ten measurements or more often if the spectrophotometer is not stable.

The turbidity blank results accounts for any particles in the extract. The turbidity blank measurement is performed on each sample at 750 nm (at this wavelength there is no absorption by pigments, so the absorption measured is due to turbidity in the extract). This measurement give the gross turbidity blank Ag_{750}. The net turbidity blank is obtained by subtracting the cell blank Ag_{750} - cb_{750}. It is better if this final value is less than 0.007 absorption unit per cm of cell length.

7.2 Measurements on sections

* Stir contents of the jar again.

* Put a Whatman GF/C filter 25 mm (or a 47 mm) on a standard laboratory plastic swinnex.

* Connect an appropriate syringe to the swinnex.

* Pour the supernatant into the syringe and collect the extract in the spectrophotometer 1 cm cell.

* Put the cell in the spectrophotometer.

* Measure the absorptions with no acidification at 665 nm, 645 nm, 630 nm and 750 nm, to give Ab_{665}^{na}, Ab_{645}^{na}, Ab_{630}^{na} and Ag_{750}^{na}.

* Acidify the extract in the cell by adding 2 drops of hydrochloric acid 0.3 $mol.l^{-1}$ with a glass rod.

* Put the cell in the spectrophotometer and wait 1 min.

* Measure the absorption on acidified extracts at 665 nm and 750 nm, to give Ab_{665}^{a} and Ag_{750}^{a}

Repeat the same procedure for each sample. Change the filter in the swinnex between each filtration, rinse the syringe with distilled water between each filtration. Rinse the spectrophotometer cell with distilled water and 90% acetone between each measurement.

8. SEDIMENT WEIGHING

Microphytobenthos biomass is usually expressed as concentration of chlorophyll *a* per dry weight of sediment.

After the spectrophotometric measurements, the sediment should be dried and weighed. The sediment weight can either be found by filtration or direct weighing of the dried sediment . For filtration the procedure is as follows:

* Leave the sediment samples overnight to evaporate the residual acetone.

* The next day, EITHER: filter as follows :

Fold a coarse Whatmann paper filter marked with the sample number and put it in a small plastic funnel.
Attach the funnel to a vacuum flask linked to a vacuum pump.

Tip the sediment into the funnel; wash the sample bottle with a small amount of distilled water to get all the sediment onto the filter.

Remove the liquid by a vacuum filtration.

Fold the filter around the sediment.

Put the sediment in an oven (100 °C) overnight to dry.

The next day, remove the sediment from the filter and weigh it on a 10 mg accuracy balance.

OR

place the sample jar in an oven at 100 °C overnight and carefully weigh the dried sediment the next day.

9. CALCULATIONS

The gross 750 nm absorption must be corrected by subtracting the cell blank from all the measurements (§ 7.1). Then, the net absorptions are obtained by subtracting the corrected measurements at 750 nm from the absorptions measured at 665 nm as follows:

- before acidification

$$A_{665}^{na} = Ab_{665}^{na} - (Ag_{750}^{na} - cb_{750})$$

$$A_{645}^{na} = (Ab_{645}^{na} - cb_{645}) - (Ag_{750}^{na} - cb_{750})$$

$$A_{665}^{na} = (Ab_{630}^{na} - cb_{630}) - (Ag_{750}^{na} - cb_{750})$$

- after acidification

$$A_{665}^{a} = Ab_{665}^{a} - (Ag_{750}^{a} - cb_{750})$$

$$[\text{chl } a]\ (\mu g.g^{-1}) = \frac{26.7\ (A_{665}^{na} - A_{665}^{a}) \times v}{l \times W}$$

$$[\text{chl } b]\ (\mu g.g^{-1}) = \frac{(-3.94\ A_{665}^{na} + 20.97\ A_{645}^{na} - 3.66\ A_{630}^{na}) \times v}{l \times W}$$

$$[\text{chl } c]\ (\mu g.g^{-1}) = \frac{(-5.53\ A_{665}^{na} - 14.81\ A_{645}^{na} + 54.22\ A_{630}^{na}) \times v}{l \times W}$$

$$[\text{Pheo a}]\ (\mu g.g^{-1}) = \frac{26.7\ ((1.7\ A_{665}^{a}) - A_{665}^{na}) \times v}{l \times W}$$

where:
v = extraction acetone volume (ml)
l = cell optic length (cm)
W = sediment dry weight (g)

If the absorption measurements are low (< 0.05 absorption unit), it would be better to use 5 cm cells and to perform the extraction in 25 ml 90% acetone.

10. EQUIPMENT NEEDED

- corers (PVC pipes with caps)
- swinnex
- sample jars
- fridge
- PERKIN ELMER double beam spectrophotometer equipped with 1 cm or 5 cm cells
- balance
- oven