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STANDARD OPERATING PROCEDURE (SOP) - 5

PROCESSING OF MACROALGAL SAMPLES

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A/ List of equipments

- ❑ 125 µm, 45 µm and 20 µm mesh size sieves
- ❑ 50 mL screw cap tubes and rack
- ❑ A thin permanent marker
- ❑ A reservoir filled with seawater collected *in situ* from sampling sites
- ❑ A squeeze bottle with filtered seawater
- ❑ A small funnel
- ❑ 2 x 100 mL graduated cylinders
- ❑ Paraffin tape
- ❑ 5 mL plastic pipettes and pump
- ❑ A notebook and a pen
- ❑ Formalin solution (37 % weight in water)
- ❑ A micropipet (P1000) with suitable disposable tips
- ❑ Plastic Pasteur pipettes
- ❑ A portable scale
- ❑ A brush (3 cm wide)

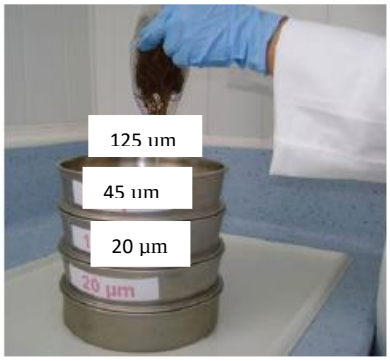
B/ Procedure

The detailed procedure can be viewed on the video tutorial available online on the CIGUAWATCH platform : https://ciguawatch.ilm.pf/ciguatera-environmental-monitoring-methodology-2/

1. SAMPLE FILTRATION

i. Stack the 3 sieves in decreasing order of mesh size (from 125 µm to 20 µm)
ii. Label the 50 mL screw cap tubes with site name, sample number, date of sampling, and fraction size, i.e. “F.45 µm” or “F.20 µm” using a permanent marker, and place them on the tube rack

iii. After creating some air and space on top of the plastic bag, vigorously shake then gently knead the macroalgal sample for 30s to dislodge microalgal cells from the macroalgal substrate
iv. Progressively pour the seawater out of the plastic bag before filtration through the nested sieves
v. To resolve clogging, hold the sieves and tap them gently until complete filtration of seawater.
vi. Fill the bag with additional seawater until the macroalgae are completely covered, then shake again and process sample as described above.
vii. Remove the 125 µm sieve
viii. Retrieve the detrital residue on the 45 µm sieve by tilting the sieve, and bring all the residual material on one side of the sieve using the squeeze bottle filled with filtered seawater
ix. Transfer the entire 45 µm fraction in the graduated cylinder using a small funnel, and bring the sample to a total volume of 40 mL



x. Cover the top of the graduated cylinder with paraffin and invert it 2-3 times to resuspend the cells, then immediately transfer the sample into the 50mL screw cap tube labelled "F.40 µm"
xi. Proceed identically with the 20 µm sieve and fraction residue using another graduated cylinder, and transfer the 20 µm fraction into the50mL screw cap tube labelled "F.20 µm"
xii. Blot the macroalgae sample on paper towel and weigh them using a portable scale
xiii. Record the species and weight of each macroalgal sample in the notebook (this value will be needed to determine the number of micro-algae cells / g of macroalgae dry weight)
xiv. Before processing the next sample, remove debris from the 125 µm mesh sieve using a small brush, and wash the sieves, funnel and graduate cylinders under tap water, then rinse with deionized water and allow to dry

2. SAMPLE PRESERVATION

i. *For routine screening/examination of samples*, preserve samples in Lugol's neutral iodine solution by adding several drops of Lugol's solution in the 15 ml tube until a light tea-color is obtained. Cap and shake to mix sample. Store the sample in the dark until microscope observation for cell enumeration. Note that Lugol's will fade with time, so it is necessary to periodically add a small amount of Lugol's solution (if the color fades away, the sample is no longer preserved !)
For long-term storage of samples, formaldehyde should be preferred as a fixative: add 1.6 mL of the formalin solution in each tube (or 38 drops using a plastic Pasteur pipette). Due to the high toxicity and carcinogenicity of formaldehyde, precautions should be taken while handling this fixative (see section "D/ Personal Protection Considerations")
ii. Seal the tubes tightly with paraffin tape
iii. Store samples at room temperature until cell enumeration under the microscope

C/ Hints

- ❑ Do not knead the macroalgal sample too vigorously to avoid lysis of micro-algal cells
- ❑ During filtration, proceed gradually to avoid spillage of the 45 µm and 20 µm fractions from sieves

- ❑ If cell isolation and culturing experiments are planned, it is imperative to remove a small aliquot (approximately 5 mL) from the 45 µm fraction sample using a plastic pipette and pump before sample preservation. In this case, carefully measure and record the final residual volume indicated on the graduated cylinder
- ❑ Microscope(s) should be kept in a cool, air-conditioned room to ensure longer lifespan of this(ese) costly equipment(s)

D/ Personal Protection Considerations

- ❑ Use a pair of safety glasses at all time
- ❑ Sample preservation: if formaldehyde is used as a fixative, work **under a laboratory fume hood or in a ventilated area**
- ❑ Sample preservation: wear gloves when handling formaldehyde and tube samples