Quantitative Periphyton Sample Preparation for Chlorophyll-a, Ash-free Dry Weight, and Taxonomic Identification

1. SCOPE AND APPLICATION

1.1 This method is based on Standard Methods 10200H. This method is used to prepare periphyton algal samples for analysis to determine the amount of chlorophyll *a* and pheophytin *a* in marine and freshwater periphyton algae by visible spectrophotometry per SOP BB-29 and to determine periphyton biomass per SOP BB-34.

1.2 This method applies to all periphyton samples with Laboratory Information Management System (LIMS) codes CHL-CORR-P and/or ALGAE-AFDW. If the sample is also scheduled for LIMS codes beginning with DTM or PRN (quantitative periphyton algal identification), coordinate with the Algal Biology Workgroup when processing the sample. An aliquot will need to be reserved for algal identification as well.

2. SUMMARY OF METHOD

2.1 Periphyton artificial substrates are brushed or scraped with an appropriate instrument for the substrate to remove all attached algae. Glass slides should be scraped using a single-edge razor blade while ceramic tiles should be brushed using a stiff brush to avoid gouging any of the tile material and adding that to the sample.

2.2 Scraped algal material is homogenized using the Maxi-Mix™ hand blender at low speed, brought up to a known volume and aliquoted for the tests that are scheduled. Sample should be constantly mixed during the aliquoting process to avoid allowing clumps of heavier algae to sink to the bottom of the container. Pour 40 mL into a 50-mL centrifuge tube for algal identification. The remainder of the sample should be immediately aliquoted for ALGAE-AFDW per SOP BB-34. Any remaining sample should be filtered for CHL-CORR-P per SOP BB-29. If the sample cannot be aliquoted immediately, pour it into a brown plastic bottle labeled with sample ID, sample volume and place it in the walk-in refrigerator.

3. APPARATUS AND EQUIPMENT

3.1 Beakers: 1 L and 250 mL

3.2 Graduated cylinders – 1 L for CHL-CORR-P and ALGAE-AFDW; 50 or 100 mL for algal identification (glassware can be rinsed between samples so only one set is needed)

3.3 Maxi-Mix™ hand blender

3.4 Plastic tray with pour spout (located on the shelf above the wash party sinks in B205)

3.5 Sample bottles – 1 brown plastic bottle each for CHL-CORR-P and ALGAE-AFDW, per sample (if sample will not be processed immediately); 1 50 mL plastic tube with orange cap per sample for algal identification

3.5.1 These bottles can be picked up from room LAB C 301, 3rd floor Receiving.

3.6 Water proof marker

3.7 Labeling tape

3.8 Scraping utensil – Small plastic brush for tiles; single-edge razor blade for glass slides

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4. REAGENTS AND CHEMICALS

4.1 Deionized (DI) water with squeeze bottle

5. SAMPLE COLLECTION AND PRESERVATION

5.1 Samples are collected, preserved, and delivered to the lab per FS 7000 – General Biological Community Sampling.

6. METHOD

6.1 Remove artificial substrates from container and place in plastic tray. Leave extra liquid and algal material in container until scraping is complete.

6.2 Scrape algae from front and back portions of the slide (omitting sides) of the artificial substrate into the 250 mL beaker, taking care to rinse off hands and utensils into the beaker with DI water during the process.

6.3 Once all the algae have been removed from the substrate (determined by visual and tactile inspection), pour the algal material from the smaller beaker into the 1 L beaker. Rinse the 250 mL beaker well with DI water and add rinse to 1 L beaker. Add material reserved in sample container to the beaker, rinse sample container well with DI, and add rinse to the beaker.

6.4 Add enough DI water to beaker to allow the hand mixer to be immersed in the sample. Lower the hand mixer into the sample so that it is not touching the bottom of the beaker. Turn the mixer on low speed and carefully work up and down (without touching bottom of beaker) until sample appears to be well-homogenized. It is important to blend slowly and control the mixer as the suction created will cause the sample to splash out of the beaker. Rinse mixer when done allowing rinse to go into beaker.

6.5 If the sample contains filamentous algae that will not break up with the hand mixer, remove the algae from the sample and place into a gridded pan. Using a razor blade or sharp scissors, snip the algal filaments into smaller, equal sections so that it will be more evenly distributed throughout the sample. Replace the cut algae back into the beaker, making sure to rinse hands and the gridded pan and add rinse to the beaker.

6.6 Once the sample is as well blended as possible, pour into a 1 L graduated cylinder, rinse the beaker well with DI water, and add that rinse to the cylinder as well. Bring final volume up to 1 L with DI water. While splitting the sample, it is important to keep the sample well mixed, so it may be necessary to pour back and forth from the cylinder to the other containers during the splitting process.

6.7 Check with the algal group to see if a separate sample was prepared. If not, separate the sample. Pour 40 mL into a labeled, plastic tube and give that container to an algal taxonomist or place in the walk-in refrigerator.

6.8 If the sample is scheduled for ALGAE-AFDW, aliquot per SOP BB-34.

6.9 For CHL-CORR-P, filter any remaining sample per SOP BB-29.

6.10 Rinse all instruments and glassware well with DI water, remove excess DI water, and proceed to next sample.

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7. SAMPLE ANALYSIS PROCEDURES

7.1 Samples are analyzed per SOP BB-29 for chlorophyll *a*, SOP AB-03 and AB-03.1 for algal identification, and SOP BB-34 for periphyton ash-free dry weight.

Note: For CHL-CORR-P samples, a p number must be calculated to two decimal places. When running the samples using the spectrophotometer, the p-number should be entered into the "volume filtered" column in the Perkin Elmer software, and the value in the "w=1/p=2" column should be changed to 2.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all logbook and bench sheet entries.

8.2 To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error. Date and initial the correction. Do not overwrite entries.

8.3 If a problem occurs during sample processing, a non-conformance report (NCR) is submitted describing the problem and the corrective action.

CALCULATIONS

Corrected chlorophyll-a :

$$\frac{26.7(664_C - 665_C)V_1}{p\# \times 4 \times 3750} \times 1000$$

($3750\ is\ the\ surface\ area\ of\ the\ front\ and\ back\ (no\ small\ edges)\ of\ a\ slide\ in\ mm^2$)

$$Where,\ p\# = \frac{volume\ filtered}{total\ volume} \times \#\ of\ slides$$

$1000\ is\ a\ conversion\ from\ volume\ to\ surface\ area\ and\ mm^2\ to\ m^2$

$$\frac{26.7(664_C - 665_C)V_1}{p\# \times 4 \times 3750} = \left(\frac{mg}{L}\right)\left(\frac{mL}{mm^2}\right)\left(\frac{L}{1000\ mL}\right)\left(\frac{mm^2}{0.000001\ m^2}\right) = \frac{mg}{m^2} \times 1000$$

P number (for CHL-CORR-P):

$$p = \frac{\text{volume filtered} \times \text{total number of slides}}{\text{total volume}}$$

Note: the surface area of a slide is 3,750 mm^2 front and back excluding sides.

9. SAFETY

9.1 Some types of algae can cause dermatitis. Use gloves and safety glasses when cleaning slides and processing sample.

9.2 Review Laboratory Health and Safety Plan.

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10. POLLUTION PREVENTION AND WASTE MANAGEMENT

10.1 Review Laboratory Health and Safety Plan.

11. REFERENCES

11.1 FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

11.2 FDEP Laboratory Health and Safety Plan http://publicfiles.dep.state.fl.us/DEAR/DEAR%20Safety/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202016.pdf

11.3 FS 7000 *General Biological Community Sampling*

11.4 DEP SOP BB-29 *Spectrophotometric Determination of Corrected and Uncorrected Chlorophyll a and Pheophytin*

11.5 DEP SOP AB-03 *Periphyton Non-Diatom Taxonomic Analysis*

11.6 DEP SOP AB-03.1 *Periphyton Diatom Species Proportional Counts*

11.7 DEP SOP BB-34 *Periphyton Biomass Using Ash-Free Dry Weight.*

APPENDIX OF CHANGES

09/08/2014	Changed storage for ALGAE-AFDW samples from refrigeration to freezing.
01/15/2015	Formatted for 508 compliance
02/19/2015	Updated to reflect storage procedures.
12/29/2016	Reformatted Section 3; changed D.I. to DI to be consistent with Section 4 and to be consistent with other SOPs; added data correction instructions and processing error instructions to Section 8.
12/20/2017	Updated links to FDEP Laboratory Quality Manual and FDEP Laboratory Health and Safety Plan in References section.
01/03/2019	Updated link to FDEP Laboratory Quality Manual.
12/19/2019	Updated method and added calculations.
12/04/2020	Clarified sample analysis and added calculations for p number.

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