ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

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IDENTIFICATION AND ENUMERATION OF SOFT-ALGAE AND ENUMERATION OF TOTAL NUMBER OF DIATOMS

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Protocol P-13-63
Identification and Enumeration of Soft-Algae and Enumeration of Total Number of Diatoms

1. PURPOSE

1.1. The U.S. Geological Survey (USGS) National Water-Quality Assessment Program (NAWQA) collects two kinds of quantitative algae samples analyzed by the Phycology Section of the Patrick Center for Environmental Research, ANSP; RTH (Richest Targeted Habitat) samples and DTH (Depositional Targeted Habitat) samples. Similar sampling protocols are used by other researchers and agencies including the U.S. National Science Foundation (NSF) funded National Ecological Observatory Network (NEON). This protocol describes a quantitative procedure for analyzing the soft-algal component of samples collected with either the DTH or RTH protocol or similar protocols.

1.2. This procedure is quantitative and designed to provide data on algal densities (cells per cm^2 or cells per bottle) and amount of algal biovolume (μm^3 per cm^2 or μm^3 per bottle).

2. SCOPE

2.1. This protocol describes procedures for identification, enumeration, documentation, and measurement of soft- algae.

2.2. This procedure is applicable to the analysis of the soft-algal component of samples collected by the DTH and RTH or similar sampling protocols of the USGS NAWQA program. A similar protocol (P-13-39) describes the procedures for analyzing the diatom component of DTH and RTH samples.

2.3. Personnel responsible for these procedures include the soft-algae analysts.

3. REFERENCES

3.1. Palmer, C.M. and T.E. Maloney. 1954. A new counting slide for nannoplankton. American Society of Limnology and Oceanography Special Publication Number 21. 6 pp.

3.2. PCER, ANSP. 2002. Analysis of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples. Protocol No. P-13-39.

3.3. Academy of Natural Sciences. 2002. Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. Charles, D.F.; Knowles, C.; and R. Davis eds. Report Number 02-06 of the Patrick Center for Environmental Resarch. 124pp.

3.4. Weber, C.I. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-73-001. National Environmental Research Center, Office of Research & Development, U. S. Environmental Protection Agency. Cincinnati, OH.

3.5. National Science Foundation, National Ecological Observatory Network. 2016. AOS Protocol and Procedure: Periphyton, Seston and Phytoplankton Sampling. NEON Document #: NEON.DOC.003045, Revision A.

4. DEFINITION

4.1. **Aliquot.** Is defined as a portion of a liquid sample or subsample.

4.2. **Fraction.** During algal analysis, an aliquot of the soft-algae subsample is used in a counting chamber. Prior to analysis, the subsample may be concentrated or diluted forming additional solutions from which an aliquot can be taken. We have adopted the term fractions to identify the specific solutions that can be analyzed in counting chambers.

4.1. **Natural counting unit**. Each natural grouping of algae (i.e., each individual filament, colony, or isolated cell) is defined as a natural counting unit. Diatoms are an exception; each diatom cell is always considered a natural counting unit, even if attached to other cells. The main purpose of using 'natural counting units' is to prevent a colonial or filamentous form from dominating a count, having more degrees of freedom for obtaining random distributions.

5. APPARATUS/EQUIPMENT

5.1. Compound microscope with 40-45x objectives for a total system magnification of 400-450x, and mechanical stage.

5.2. Palmer-Maloney Counting Cells with ceramic chamber, chamber depth of 0.4 mm and volume of 0.1 ml.

5.3. Glass microscope cover slips, rectangular, 22 x 50 mm, #1 thickness.

5.4. Pasteur pipettes, 5.25 inches.

*5.*5. Glycerin, analytical grade.

5.6. "Tabulator" and "BioVol" programs (Visual Basic applications installed on analysts computer).

6. METHODS

6.1. Prepare Palmer-Maloney counting cell.

6.1.1. Place a rectangular cover slip (#1 thickness, 22 x 50 mm) at the top of the counting cell, covering about 1/3 of the chamber, but not across the center of the cell.

6.1.3. Thoroughly mix the Palmer-Maloney fraction and draw it into an elongated Pasteur pipette (5.25 inch; about 1-1.3mm inside diameter). Quickly add the fraction drop-wise into the center of the chamber. When the surface tension starts to draw the cover slip across the chamber, adjust the sides of the cover slip so that the ends of the chamber are covered and the cover slip hangs over both sides of the ceramic portion of the counting cell.

6.1.4. Add glycerin to the area where the cover slip hangs over the ceramic portion. This seals the cover slip to the counting cell temporarily. Without excess heat or vibration, the counting cell can be used for a week or more.

6.2. **Choose to count random fields or along transects.** Select one of the two options below. Neither one is preferred over the other.

6.2.1. **Determine random fields:** Using a high dry microscope objective (40-45x objective, 400-450x total system magnification), identify and enumerate algae in selected, random fields. Prior to enumerations, scan the counting chamber and determine the approximate number of transects or fields needed to enumerate 300 natural units (minimum of 10 and maximum of 100 random fields). Starting at a random starting place in the upper left-hand quadrant develop a pattern that allows for equal probability of landing in any area of the cell or chamber with the exception of the edges and the center. A pattern with a maximum of 50 fields is made by having a grid of 8 x 8, subtracting 3 or 4 fields in either direction of the center. .

6.2.2. Determine transects: Using a high dry microscope objective (40-45x objective, 400-450x total system magnification) with a calibrated stage, identify and enumerate algae along transects, either horizontally or vertically across the chamber of the Palmer-Maloney cell. Without looking into the microscope, choose a location near the left edge in the upper third of the chamber (if vertical transects are analyzed, choose a location near the top edge in the left third of the chamber). Make a transect by moving only the horizontal stage control (or vertical control for vertical transects) a measured distance. Develop a pattern for the transects that will avoid the center and edges of the chamber. A second Palmer-Maloney cell can be used, if necessary (300 natural units need to be counted with a minimum of three complete transects).

6.3. **Enumerate 300 natural counting units.** With the exception of time spent learning new floras, this analysis should be finished in approximately 2-3 and no more than 4 hours.

6.3.1. Using the pattern developed above (section *6.2.*), move the microscope stage to a new position in the pattern. Make all movements of the microscope stage without looking through the objectives.

6.3.2. **Identify and enumerate all algal forms in the field of view:** Enumerate algal forms using natural counting units. Natural units are defined as one for each colony, filament, diatom cell (regardless if colonial or filamentous) or unicell. With the exception of diatoms, identify algal forms to the lowest possible taxonomic level.

6.3.3. Categorize diatoms as either "living" or "dead" at the time of collection, and quantify them separately. If there is any protoplast material in the frustule, the diatom is considered to have been living when collected.

6.3.4. Count the number of algal cells comprising each multicellular counting unit.

6.3.5. Tabulate the data on a bench sheet, mechanical tabulator or into a computer application (eg.,ANS developed "Tabulator").

6.3.6. Repeat steps *6.3.1, 6.3.2* and *6.3.4* until 300 natural counting units have been enumerated. Count only "living" diatoms as part of the required 300 natural algal units.

6.3.7. Record the number of fields or total length of the transect for the area that was observed.

6.4. **Measure cell biovolumes**. For each group of samples, measure the dimensions of the taxa that contribute most to sample biovolume.

6.4.1. Determine the taxa that need biovolume measurements by listing all the species in the samples that have accounted for 5% or more of a sample count. Study protocols might require more or less taxa that should be measured.

6.4.2. For each taxon requiring biovolume measurements, select a simple geometric figure matching the shape of the taxon as best as possible, and determine the dimensions that must be measured. Record this information on a bench sheet or in a computer application (eg., ANS developed "BioVol"). Measure and record the dimensions of at least five specimens. If these measurements are not in the range of previous measurements, measure additional specimens until 15 specimens have been measured from the particular study. No more that five specimens should be measured from a single sample.

6.5. **Calculate cell densities.** The calculation of periphyton abundance is made by considering how much volume was scanned in the Palmer-Maloney cell, applying dilution concentation factors of samples, subsamples and fractions and multiplying by the area (or volume if per bottle) that was sampled. Biovolume values are determined by multiplying the cell density (cells/mm^2 or cells/bottle) by the average biovolume of each cell (μm^3).

6.5.1. Periphyton abundance (cells/mm^2) is calculated as follows:

$$\text{cells/cm}^2 = \frac{\text{cell count} * \text{field DCF} * \text{subsample DCF} * \text{fraction DCF} * \text{sample volume(ml)}}{\text{volume scanned (ml)} * \text{area sampled (cm}^2\text{)}}$$

where:

cell count = number of cells enumerated

DCF = dilution concentration factor

If transects: volume scanned (ml) = total scan length (cm) * microscope field diameter (cm) * depth counting cell (cm);

If random fields: volume scanned (ml) = microscope field volume (cm3) * number of fields scanned.