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IDENTIFICATION AND ENUMERATION OF ALGAE IN PHYTOPLANKTON SAMPLES

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Protocol P-13-52

Identification and Enumeration of Algae in Phytoplankton Samples

1. PURPOSE

1.1. Phytoplankton is used in biological assessment and monitoring studies by several state and federal agencies. These procedures describe methods that utilize whole-water phytoplankton samples collected from lentic (lakes and ponds) and lotic (rivers and streams). This protocol describes quantitative procedures for analyzing the soft-algal component of phytoplankton and counting the total number of diatoms.

1.2. This procedure is quantitative and designed to provide data on algal densities (as cells per ml) and amount of algal biovolume (μm^3 per ml) at a sampling site. Another protocol (P-13-39) describes the procedures for identifying and enumerating the diatom component of phytoplankton samples.

2. SCOPE

2.1. This protocol is applicable to the analysis of the soft-algal component of whole-water phytoplankton samples. It includes procedures for identification (to lowest possible taxon level) and enumeration of algal species, taking measurements of the dimensions of algal species for biovolume determinations.

2.2. Personnel responsible for these procedures include sample preparers, phytoplankton analysts and those involved with data entry.

2.3. Two methods of analyzing phytoplanktonic algae are described: 1) using Utermöhl sedimentation technique (Hasle 1978) with analysis on an inverted microscope, and 2) analyzing in counting cells (Palmer and Maloney 1954) of sedimentation concentrated samples.

3. REFERENCES

3.1. Hasle, G.R. 1978. The inverted-microscope method. Chapter 5.2.1 in Phytoplankton Manual. A. Sournia, ed. United Nations Educational, Scientific and Cultural Organization. Paris. 337 pp.

3.2. 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.

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

3.4. PCER, ANSP. 2002. Preparation of Algal Samples for Analysis Using Palmer-Maloney Cells. Protocol P-13-50.

3.5. PCER, ANSP. 2002. Subsampling Procedures for USGS NAWQA Program Periphyton Samples. Protocol P-13-48.

3.6. 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.

3.7. 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.

4. DEFINITIONS

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 (use of Palmer-Maloney and Utermöhl chambers are described here). 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.

4.3. **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 10-15x, 40-50x and 90-100x objectives. Objectives are mounted below the stage for the inverted microscope method.

5.2. Settling chambers with 10-ml to 100-ml settling tubes. There are a couple of basic varieties of the Utermöhl sedimentation chambers (1) the tubular variety (usually 10-25ml) consisting of a threaded, fitted base with a round base plate and (2) the combined plate chamber variety consisting of sedimentation tubes from 10-100ml, a plexiglass base unit with a round base plate. The base plates are glass microscope coverslips (#1, 25.1-27.5mm diameter). A thick glass (0.2 mm) is used as a cover plate..

5.3. Palmer-Maloney counting cells with ceramic chamber, chamber depth of 0.4 mm and volume of 0.1 ml. The counting cells use rectangular glass microscope cover slips (#1, 22x50mm).

5.4. 20-ml vials with screw top caps.

5.5. Pasteur pipettes, 5.25 inches.

5.6. Glycerin, analytical grade.

6. METHODS

6.1. **Choose Analysis Method.** Both analysis methods, inverted microscope and Palmer-Maloney counting cell, result in similar counts when used correctly. The inverted scope is better when the original sample volume is limited (i.e., less than 400 ml) because high numbers of cells are needed for the Palmer-Maloney counts. Detritus, a problem with both techniques, is especially troublesome for the inverted microscope method.

6.2. **Pre-Concentrate Subsamples.** For both techniques, the original sample should be concentrated prior to adding to a counting chamber.. This initial concentration should be approximately 10 times the original whole-water sample, leaving about 20 ml of concentrate for analysis. Samples are concentrated by a combination of settling in tall glass cylinders (settle for at least 24hrs) and by centrifugation (1000 g for 20 min). Determine the amount of concentration and calculate a dilution concentration factor [DCF] by dividing the final volume (this is designated as fraction volume) by the beginning volume (this was the volume of the subsample). The subsample volume, fraction volume and subsample DCF should be recorded.

6.3. **Prepare Palmer-Maloney Counting Cell.**

6.3.1. Prior to using an aliquot of the fraction to fill a Palmer-Maloney cell, the concentrated fraction may need further preparation as described in Protocol P-13-50).

6.3.2. 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.

6.3.3. Thoroughly mix the Palmer-Maloney fraction and draw it into an elongated Pasteur pipette (5.25 inch). 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. Then add glycerin to the area where the cover slip hangs over the ceramic portion. This seals the cover slip to the counting cell; without excess heat or vibration, the counting cell can be used for a week or more.

6.4. **Prepare Utermöhl Sedimentation Chamber.**

6.4.1. Attach a glass cover glass to the bottom of an Utermöhl sedimentation chamber. For tubular varieties of settling chambers, seal a cover glass to the threaded end of the tube and screw the tube into the base assembly. Assemble the plate chamber type of settling chambers by sealing a cover glass on the bottom of the base, locking it into place with the metal ring, and sealing the cylinder on top of the base. Use a light amount of vacuum grease to seal the cover glasses and cylinders. It is critical that the cover glass be clean and grease-free.

6.4.2. Homogenize the concentrated samples by repeatedly inverting the sample bottle. Place a 10-ml aliquot of the sample into the assembled settling chamber.

6.4.3. Let the sample settle for at least 8 hours.

6.4.4. For the plate chamber type of Utermöhl chamber, drain the volumetric cylinder by sliding over the drainage hole. Slide the cover plate over the chamber without allowing air bubbles to form. Analysis should proceed within a few hours of removing the cylinder.

6.5. **Choose to count random fields or along transects.** Both methods (inverted microscope and Palmer-Maloney counting cell) involve counting phytoplankton cells in a chamber, by counting either random fields or along transects. Choose one of the following.

6.5.1. 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 500 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. For Palmer-Maloney cells, a maximum pattern with 50 fields is made by having a grid of 8 x 8, subtracting 3 or 4 fields in either direction of the center. For the Utermöhl sedimentation chamber, a maximum pattern with 100 fields is made by having a grid of 12 x 12, subtracting 3 or 4 fields in either direction of the center.

6.5.2. 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 or Utermöhl plate chamber. 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 or Utermöhl chamber can be used, if necessary (500 natural units need to be counted with a minimum of three complete transects).

6.6. **Enumerate 500 natural algal units.**

6.6.1. Using the pattern developed above (section *6.5.*), move the microscope stage to a new position in the pattern. Make all movements of the microscope stage without looking through the objectives.

6.6.2. Identify and enumerate all algal forms in the field of view: Enumerate algal forms using natural counting units. Natural counting 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. Enumerate diatoms as "live" if any protoplast can be observed or "dead" if the frustule is empty.

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

6.6.4. Tabulate the data either on a bench sheet, mechanical tabulator or direct entry computer program (eg., ANS developed "Tabulator").

6.6.5. Repeat steps *6.6.1. through 6.6.4.* until 500 natural algal units have been enumerated. Count only "live" diatoms as part of the required 500 natural algal units.

6.6.6. Record the number of fields or total length of the transect that was observed.

6.7. **Enumerate larger, rarer taxa.** There is an additional, procedure used for samples with low concentrations (less than five natural counting units) of large cells or colonies. Using a low-power objective (10-15x), scan 20 fields or 4 transects. Count only the larger, rarer taxa (ie., those less than 10 natural units in high-power count) Similar to the high-power count, enumerate natural units and estimate the number of cells.

6.8. **Measure cell biovolumes.** For each group of samples, determine the biovolume of the algal taxa that contribute most to sample biovolume.

6.8.1. Determine the taxa that need biovolume measurements by listing all the species in the samples that accounted for 5% or more of a sample count (i.e., 25 or more natural units of the 500 natural units enumerated).

6.8.2. 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. Data can be entered into computer files via specialized applications or through spreadsheets.

6.8.3. 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 study area. No more than five specimens should be measured from a single sample.

6.10. **Calculation of phytoplankton abundances and biovolumes.** The calculation of phytoplankton abundance depends on the apparatus used during analysis. Biovolume values are determined by multiplying the abundance (cells/ml) by the average biovolume of each cell (μm^3). The average biovolume of each cell is determined by averaging all values for the taxon. Equations for abundance calculations are given below.

6.10.1. If the inverted microscope method was used in the analyses, phytoplankton abundance (cells/ml) is calculated as follows:

$$\text{cells/ml} = \frac{\text{cell count} * \text{field DCF} * \text{subsample DCF} * \text{fraction DCF} * \text{chamber area } (mm^2)}{\text{area scanned } (mm^2) * \text{chamber vol (ml) used}}$$

where:

cell count = number of cells enumerated

DCF = dilution concentration factor

If transects were used: area scanned (mm^2) = total scan length (mm) * microscope field diameter (mm);

If random fields were used: area scanned (mm^2) = microscope field area (mm^2) * number of fields scanned.

6.10.2. If Palmer-Maloney counting cells were used in the analyses, phytoplankton abundance (cells/ml) is calculated as follows:

$$\text{cells/ml} = \frac{\text{cell count} * \text{field DCF} * \text{subsample DCF} * \text{fraction DCF}}{\text{volume scanned (ml)}}$$

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 of chamber (cm)

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