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Techniques of Water-Resources Investigations
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Chapter A4

# METHODS FOR COLLECTION AND ANALYSIS OF AQUATIC BIOLOGICAL AND MICROBIOLOGICAL SAMPLES

L.J. Britton and P.E. Greeson, Editors

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LABORATORY ANALYSIS

# SESTON
# (TOTAL SUSPENDED MATTER)

## Introduction

The weight of suspended matter in water (seston) is an important measurement in ecological studies. For example, this value has been shown to correlate with optical properties (Jerlov, 1968) and with temporal and spatial changes in aquatic environments (Maciolek and Tunzi, 1968; Moss, 1970; Reed and Reed, 1970). For some analyses, the sample may be prefiltered through a 150- to 350-$\mu$m mesh to eliminate large particles before filtration. The particulate residue remaining in the sample after sieving is designated microseston.

## Collection

The sample-collection method will be determined by the study objectives. In lakes, reservoirs, deep rivers, and estuaries, seston abundance may vary transversely and with depth (Patten and others, 1966). To collect a sample of the seston at a particular depth, use a water-sampling bottle, Van-Dorn type (fig. 11). To collect a sample representative of the entire flow of a stream, use a depth-integrating sampler (Guy and Norman, 1970; Goerlitz and Brown, 1972). For small streams, a depth-integrated sample or a point sample at a single transverse position located at the centroid of flow may be adequate. Study design, collection, and sampling statistics for streams, rivers, and lakes are described in Federal Working Group on Pest Management (1974).

Seston samples need to be filtered immediately after collection. Record the mesh size of prefilter, if used. Record the volume of water filtered. The filters need to be thoroughly dried or stored in tightly closed plastic petri dishes at 1 to 4 °C (do not freeze) until ovendried. Samples that cannot be filtered without delay need to be preserved using 40 mg mercury per liter. Preservation will stabilize the seston content of samples for at least 8 days. However, the results of analyses of preserved samples are not necessarily the same as those obtained by immediate filtration.

The method described in this chapter is the glass-fiber filter adaptation by Strickland and Parsons (1968) of the method developed by Banse and others (1963).

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# Glass-fiber filter method

## (B-3401-85)

**Parameters and Codes:**
**Seston, dry weight (mg/L): 71100**
**Seston, ash weight (mg/L): 71101**

**1. Applications**

The method is suitable for all water.

**2. Summary of method**

A known volume of water is prefiltered through a tared glass-fiber filter to remove the particulate matter. The increase in weight of the filter after drying at 105 °C is a measure of the dry weight of particulate matter in the sample. After ashing the residue at 500 °C, the difference between dry weight and ash weight is the weight of particulate organic matter in the sample.

**3. Interferences**

Although the method generally is free from interferences, bottles and sampling equipment need to be clean, and samples, filters, and funnels need to be protected from dust. Filtration needs to be at decreased pressure to avoid rupture and loss of cell contents of fragile organisms. Saline samples need to have the salts washed from the filter residues to prevent erroneous weight values.

**4. Apparatus**

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Aluminum foil*, laboratory grade.

4.2 *Balance*, capable of weighing to at least 0.1 mg.

4.3 *Desiccator*, containing silica gel or anhydrous calcium sulfate.

4.4 *Drying oven*, thermostatically controlled for use at 105 °C.

4.5 *Filter flask*, 1 L or 2 L. For onsite use, a polypropylene flask is appropriate.

4.6 *Filter funnel*, vacuum, 1.2-L capacity, stainless steel.

4.7 *Forceps*, stainless steel, smooth tip.

4.8 *Glass filters*, 47-mm diameter disks. For best results, all filters for a series of samples, including control filters, need to be from the same box and need to have a tare weight of 70- to 100-mg ($\pm$10 mg) weights.

4.9 *Graduated cylinders*, plastic, of sufficient capacity (100 and 500 mL and 1 L are convenient sizes) for measuring known volumes of water samples.

4.10 *Manostat* that contains mercury and calibration equipment to regulate the filtration suction at not more than 300 to 350 mm of mercury when filtering using an aspirator or an electric vacuum pump.

4.11 *Muffle furnace*, for use at 500 °C.

4.12 *Plastic petri dishes* and *covers* for filter storage.

4.13 *Sample containers*, plastic bottles, 1- to 5-L capacity.

4.14 *Vacuum pump*, water-aspirator pump or an electric vacuum pump for laboratory use; a hand-operated vacuum pump and gauge for onsite use.

4.15 *Water-sampling bottle*, Van-Dorn type. Depth-integrating samplers are described in Guy and Norman (1970).

**5. Reagents**

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Distilled or deionized water*. Filter if in doubt about whether water is particle free.

5.2 *Mercuric chloride solution*, 1 mL=40 mg mercury ($Hg^{2+}$). Dissolve 55 g mercuric chloride ($HgCl_2$) in distilled water and dilute to 1 L.

**6. Analysis**

6.1 Arrange the required number of glass filters (do not overlap) on the shiny side of aluminum foil and heat in a muffle furnace at 450 to 500 °C for 30 minutes. Do not allow the temperature to exceed 500 °C. This preparation hardens the filters and removes any organic matter. About 20 filters is a convenient number with which to work.

6.2 Use at least 10 percent of the filters as controls. For large batches, use every 10th filter as a control; for small batches, use a filter at the beginning and one at the end as controls. The treatment of control filters is identical to that of the test filters except that no water is filtered through them.

6.3 Cool and transfer all filters, including the controls, to a shallow container of distilled water for 5 minutes. Use about 100 mL water for each filter. Handle the filters very carefully using clean, smooth-tip forceps to avoid fraying the filters.

6.4 Using the forceps, transfer the filters to the shiny side of the aluminum foil after gently shaking off excess water. Dry the filters in an oven at 105 °C for 30 minutes. Cool to room temperature in a desiccator (Note 1).

Note 1: Because of the difficulty of marking glass filters, the individual filters should be accounted for throughout the remaining steps. The filters should be placed on the aluminum foil in a definite sequence and, whenever possible, each filter should be kept in a numbered plastic petri dish.

6.5 Weigh each filter to the nearest 0.1 mg as rapidly as

possible, and record this initial (tare) weight value. Close the desiccator tightly after each removal. Store the tared filters in numbered plastic petri dishes until needed.

6.6 When a sample is to be filtered, place a tared filter, wrinkled surface upward, on a filter funnel. A small slip of aluminum foil under the edge of the filter facilitates removal of the wet filters.

6.7 When vacuum is applied, wet the filter using distilled water to seat the disk on the filter funnel.

6.8 Measure out a suitable quantity of thoroughly mixed sample into a graduated cylinder. Complete mixing of the sample is essential prior to measuring. Pour the sample into the filter funnel and filter using a manostat or other suitable method to keep vacuum to 300 to 350 mm mercury (about 6 psi).

6.9 Maintaining vacuum, wash the funnel and filter three times using 5- to 10-mL volumes of distilled water, allowing the filter to suck "dry" between each wash.

6.10 Disconnect the vacuum and, using smooth-tip forceps, place the wet filter on the shiny side of aluminum foil. Store the filters at 1 to 4 °C in numbered petri dishes at this stage, if necessary.

6.11 Dry the filters in an oven at 105 °C for 1 hour. Include at least two control filters from 6.5 in this drying step for each batch of sample filters.

6.12 Place the filters in a desiccator, cool, and reweigh each filter rapidly to the nearest 0.1 mg as in 6.5. Include the control filters from 6.11. Use these values to calculate dry weight.

6.13 Again place the filters that have dried residue and the control filters on the shiny side of aluminum foil and heat in a muffle furnace at 500 °C to constant weight. Heat at least 30 minutes, but some samples may require longer times. Cool and rewet the filters using distilled water to restore the water of hydration of clays and other minerals that may have been lost.

6.14 Place the filters in a desiccator and reweigh each filter rapidly to the nearest 0.1 mg as in 6.5. Include the control filters from 6.13. These values are used to calculate ash weight.

## 7. Calculations

7.1 Dry weight of seston (milligrams per liter)

$$= \frac{\begin{array}{c}\text{Dry weight of filter and residue (milligrams)} \\ - \text{ Tare weight of filter (milligrams)}\end{array}}{\begin{array}{c}\text{Volume of water sample (liters)} \\ - \text{ Blank correction (milligrams)}\end{array}},$$

where blank correction (milligrams) = mean weight of control filters, in milligrams (from 6.12) − mean weight of control filters, in milligrams (from 6.5). The blank correction value may be positive or negative but should not exceed about 0.5 mg.

7.2 Ash weight of seston (milligrams per liter)

$$= \frac{\begin{array}{c}\text{Ash weight of filter and residue (milligrams)} \\ - \text{ Tare weight of filter (milligrams)}\end{array}}{\begin{array}{c}\text{Volume of water sample (liters)} \\ - \text{ Blank correction (milligrams)}\end{array}},$$

where blank correction (milligrams) = mean weight of control filters, in milligrams (from 6.14) − mean weight of control filters, in milligrams (from 6.5). The blank correction value may be positive or negative but should not exceed about 0.5 mg.

7.3 Ash-free or organic weight of seston (milligrams per liter) = dry weight of seston (milligrams per liter) − ash weight of seston (milligrams per liter).

## 8. Reporting of results

Report seston as follows: less than 1 mg/L, one significant figure; 1 mg/L or greater, two significant figures.

## 9. Precision

No numerical precision data are available.

## 10. Source of information

Guy, H.P., and Norman, V.W., 1970, Field methods for measurement of fluvial sediment: U.S. Geological Survey Techniques of Water-Resources Investigations, bk. 3, chap. C2, 59 p.

# PERIPHYTON

## Introduction

Periphyton literally refers to aquatic plants growing around (on) solid surfaces. European investigators originated the term about 1924 to describe organisms growing on artificial substrates in water (Cooke, 1956). Recently, the term "periphyton" has been extended to include the entire community of micro-organisms that live attached to or on solid submerged surfaces, generally above the depth of light extinction (Young, 1945; Sladecek and Sladeckova, 1964; Wetzel, 1964). The term encompasses not only algae but associated bacteria, fungi, protozoans, rotifers, and other small organisms. Although some of the latter are more accurately benthos, they are invariably sampled as part of the community by most methods. Thus, the methods of periphyton estimation that follow include both autotrophic and heterotrophic components of the periphyton unless otherwise stated. Periphyton is synonymous with the term "Aufwuchs," as described by Ruttner (1963): "***all those organisms that are firmly attached to a substratum but do not penetrate into it." The complexity of the periphyton community has spawned an equally complex terminology based on substrate classification, and the reader is referred to Weitzel (1979) for a more complete account.

## Collection

Most analyses of the periphyton community have been adopted from long-established methods of phytoplankton analyses. The attached benthic nature of periphyton, however, presents special collection problems that directly affect the success of various estimates. In fact, problems related to sampling are the principal sources of error in most methods. Major sampling problems include adherence of the periphyton to mineral substrates and the patchiness of their distribution, particularly in lotic systems. Gravel substrates, even those which seem smooth and uniform, actually have a complex and irregular texture. Methods have been developed for collecting periphyton from natural substrates (Douglas, 1958; Ertl, 1971; Stockner and Armstrong, 1971), which usually are restricted to taxonomic studies or community-structure analysis. However, biomass and production estimates are derived more commonly from artificial substrates (Nielson, 1953; Grzenda and Brehmer, 1960; Maciolek and Kennedy, 1964; Neal and others, 1967; Peters and others, 1968; Tilley and Haushild, 1975a, b; Busch, 1978; Clark and others, 1979; Hoffman and Horne, 1980). The decision to use natural or artificial substrates should be considered carefully, based on the study objectives developed prior to beginning onsite investigations.

Careful sampling of natural substrates is likely to yield more complete information on species composition because irregularities of the microhabitat will be incorporated into the sample. Inability to remove tissue efficiently from natural substrates, however, may produce a large underestimate of biomass. Artificial substrates enable more efficient collecting of a large number of samples and partially overcome the problem of adherence. Lack of microhabitat diversity, however, may affect patterns of colonization and biomass accumulation. Artificial substrates standardize the physical environment in studies where surface uniformity is an important consideration.

Once the decision about substrate type has been made, the inherent patchiness of periphyton distribution still needs to be considered. Because periphyton colonization is affected by numerous variables (light, depth, current velocity, and substrate texture), variability on natural and artificial substrates generally is large. Tilley and Haushild (1975a,b) reported that 21 glass microscope slides exposed for 2 weeks at a single site in the Duwamish River, Wash., had chlorophyll concentrations ranging from 1.33 to 2.81 mg/m$^2$ and a mean of 1.97 mg/m$^2$. The 95-percent confidence limit (approximated by two standard deviations) was 0.74 mg/m$^2$. Twenty-two slides exposed for 3 weeks at a single site in the Duwamish River had chlorophyll concentrations ranging from 1.89 to 4.86 mg/m$^2$ and a mean of 3.44 mg/m$^2$. The 95-percent confidence limit (approximated by two standard deviations) was 1.44 mg/m$^2$. Similarly, Pryfogle and Lowe (1979) reported differences in periphyton cell counts as large as an order of magnitude between adjacent stones in Tymochtee Creek, Ohio.

Effort always should be made to minimize possible variance by sampling habitats that are representative of the site and needs to include depth, current velocity, and canopy cover. If specific habitats are selected for comparative studies (pools, riffles), care should be taken to duplicate this habitat type at all sites, and the habitat type should be reported as well as the results. Unless care is taken to standardize the habitat, the results will indicate differences in substrate placement and collection, rather than differences in water quality.

Sufficient colonization time is another important consideration, especially for studies assessing species composition, because incubated substrates may undergo algal succession (Busch, 1978). If there is sufficient colonization time, species composition on artificial substrates generally is similar to the

natural community (Patrick and others, 1954; Castenholz, 1960; Weitzel and others, 1979; Hoffman and Horne, 1980), but large differences in biomass or chlorophyll concentrations may be measured (Grzenda and Brehmer, 1960; Castenholz, 1961; Sladeckova, 1962; Pieczynska and Spodniewska, 1963; Weitzel, 1979). Proper colonization time will depend on season, water temperature, light, and nutrient availability, and other factors. Neal and others (1967) reported that maximum accumulation of periphyton biomass on polyethylene strips occurred in about 2 weeks. Patrick and others (1954) reported a 2-week colonization period also maximized the number of species. For most circumstances, colonization period should be at least 14 days, but this will vary and must be determined for each season and water type.

Other mechanisms for overcoming the problems of patchiness are to increase the number of samples or to have larger composite samples representing a diversity of habitats at a single site. Vandalism is a common problem, so substrates need to be placed away from frequently visited areas.

## Sampling from natural substrates

Natural submerged substrates commonly contain periphyton, and a known area can be sampled quantitatively. If the area is unknown, periphyton scraped from natural substrates may be used for species identification and for determination of relative abundance. Several devices for removing periphyton from a known area of natural substrates are shown in figure 18. The instrument used by Douglas (1958) consists of a broad-necked polyethylene bottle that has the bottom removed (fig. 18*A*). The neck of the bottle is held tightly against the surface to be sampled, and the periphyton inside the enclosed area is dislodged from the substrate using a stiff nylon brush. The loose periphyton is removed from the bottle using a pipet. Ertl's (1971) device consists of two concentric metal or plastic cylinders separated by spacers (fig. 18*B*). The space between the cylinders is filled with modeling clay, and the sampler is pressed firmly against the substrate to be sampled. Using a blunt stick or metal rod, the clay is forced down onto the substrate to isolate the sampling area of the inner circle. The periphyton within the inner circle is dislodged using a stiff brush and removed using a pipet. Stockner and Armstrong (1971) sampled periphyton using a plastic hypodermic syringe that has a toothbrush attached to the end of the syringe piston (fig. 18*C*). The barrel of the syringe is held tightly against the substrate, and the piston is pushed in until the brush contacts the periphyton. The piston then is rotated several times to dislodge the periphyton and then is withdrawn pulling the periphyton up with it. A glass plate is placed immediately under the end of the barrel and the syringe inverted. Four small holes at the base of the syringe enable free movement of water when procuring the sample.

## Sampling from artificial substrates

Suitable artificial substrates are attached to supports and placed in a stream or lake (figs. 19, 20). The substrates must be submerged but may be near the surface or at any appropriate depth. In lakes, substrates commonly are suspended at several depths (fig. 19*A*, *B*, *C*) to provide a more realistic representation of the periphyton community. Substrates should be oriented similarly at all sites because settling of organic and inorganic detritus may increase depending on the orientation of the substrate (Castenholz, 1960; Liaw and MacCrimmon, 1978). Vertical orientation is preferred because it decreases the settling problem. In lakes and streams, substrates may be attached to natural objects, such as submerged trees, stumps (fig. 19*D*), logs or boulders, or

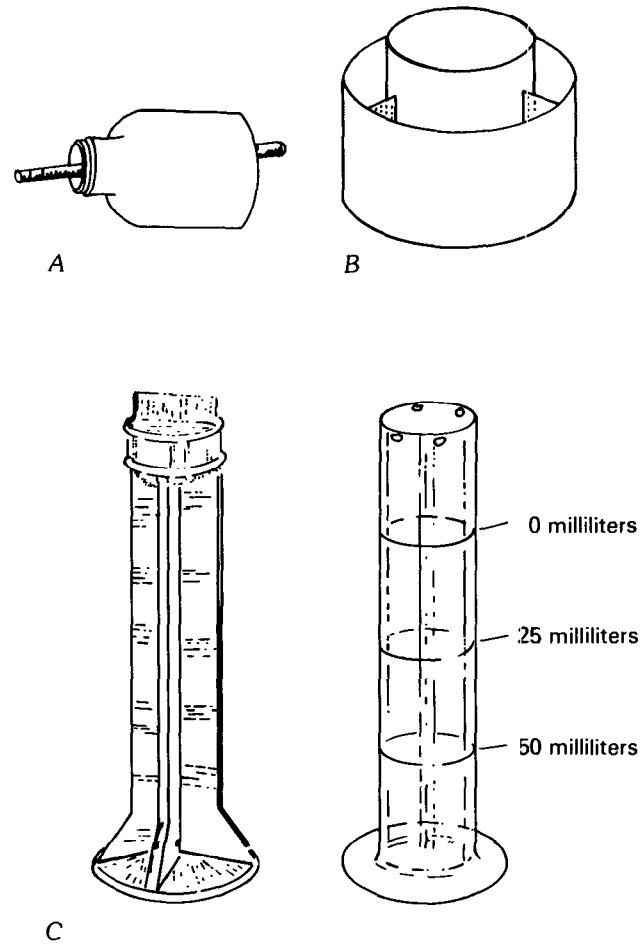


Figure 18.—Devices for collecting periphyton from natural substrates: (*A*) Brush and polyethylene-bottle device (modified from Douglas, 1958, p. 297; reproduced by permission of Duke University Press, Durham, N.C.). (*B*) Plastic or metal cylinder device (redrawn from Ertl, 1971, p. 576). (C) Plastic hypodermic syringe device (redrawn from Stockner and Armstrong, 1971, p. 218).

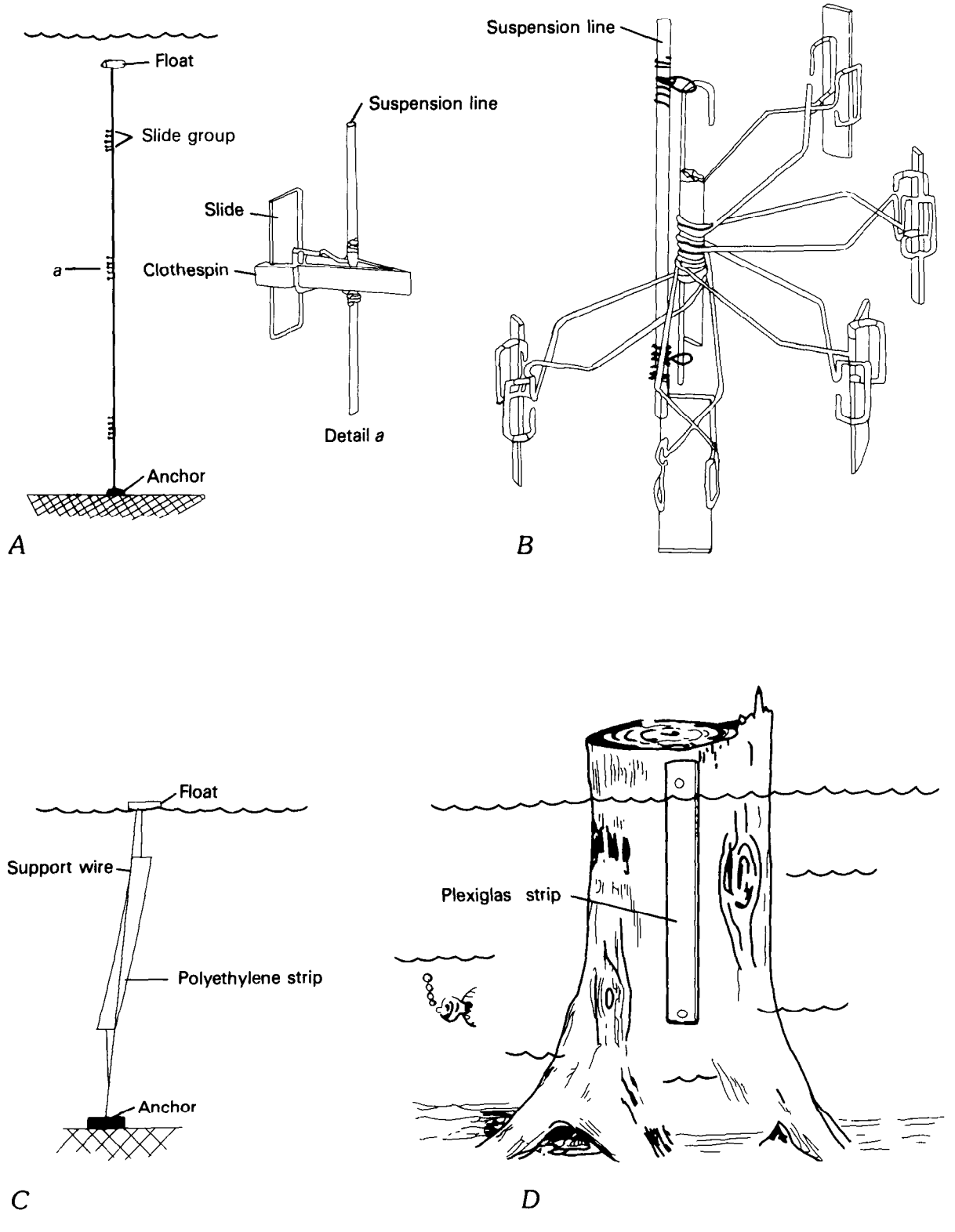


Figure 19.—Artificial-substrate sampling devices for periphyton: (*A*) Microscope slide-suspension device made from spring clothespins (from Nielson, 1953, p. 99). (*B*) Microscope slide-suspension device made from test-tube clamps (from Maciolek and Kennedy, 1964). (*C*) Polyethylene strip device. (*D*) Plexiglas strip attached to submerged object.

they may be attached to stakes driven into the bottom (fig. 20*A*). Floating samplers also may be used (fig. 20*B*), but care should be taken to allow for overestimation when water levels vary. The sampler should be secured so it will not drift into any obstruction or become beached. In extremely shallow streams, it may be necessary to construct a weir to guarantee sufficient water to float the sampler. If such a weir is constructed, data from the sample should be compared only with data obtained from comparably placed samplers. A floating sampler should not be used for any area in which there is intermittent flow for any period during the exposure time.

The artificial substrates should be placed in lighting conditions that typify the streams, rivers, or lakes being studied. For example, if most of the stream is shaded, an area that receives a great deal of sunlight should not be selected as being representative. In general, substrates collected from similar lighting conditions should be compared; but, depending on the study objective, this is not a requirement.

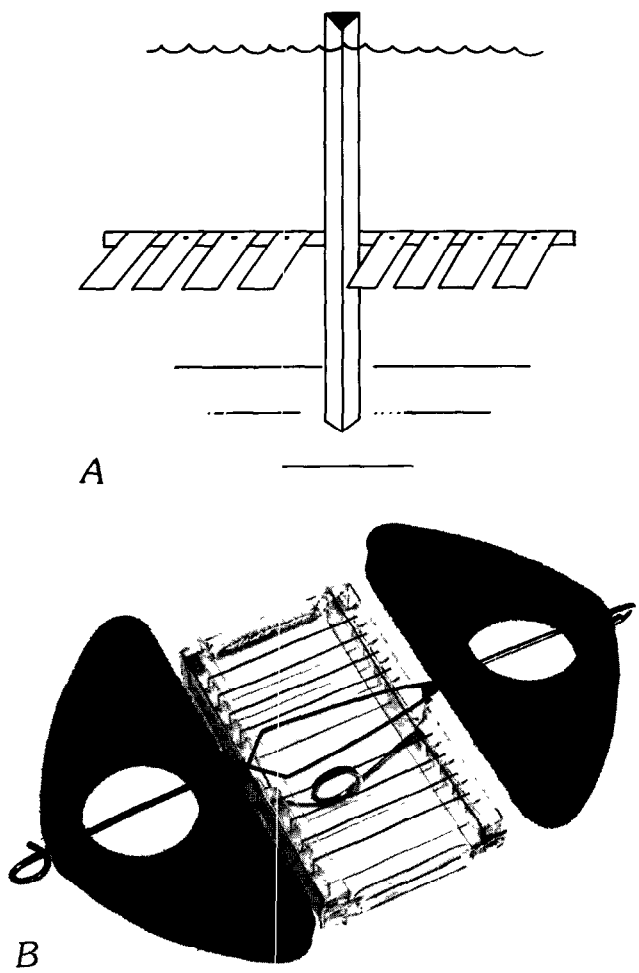


Figure 20.—Artificial-substrate sampling devices for periphyton: (*A*) Plexiglas plates attached to support (from Peters and others, 1968, p. 12). (*B*) Floating sampler, Periphytometer. (Photograph courtesy of Design Alliance, Inc., Cincinnati, Ohio.)

To ensure a continuous period of uniform colonization time of the artificial substrate, the substrate should be examined, periodically if possible, for any evidence of fouling or mechanical damage. If the substrate has been fouled or beached, the data for that sampling period should not be compared with data from any other substrate that has free, continuous, and uninterrupted exposure to the aquatic environment.

The length of time required for colonization of the substrates by periphyton will depend on other environmental factors as well as water quality. Colonization times will vary and must be determined for each season and water type. The colonization period should be sufficiently long to enable the development of a microbial community large enough for measurement and, at the same time, avoid so much growth that sloughing would occur. Test samplers can be placed prior to the actual monitoring period to determine the most desirable colonization time for the prevailing (that is, seasonal and environmental) conditions. Suggested colonization periods for fresh to brackish water, mesotrophic to eutrophic, within the thermal range of 15 to 35 °C, is 14 days. Colonization periods during low productivity (that is, lack of nutrients or low temperature) or very high productivity may, by experience, be adjusted for the onsite conditions. Colonization periods should be identical for all sites in the entire study area.

After sufficient colonization of periphyton, indicated by visible green or brown growth, remove artificial substrates from the water. Periphyton may be scraped from the substrate onsite or in the laboratory, using razor blades, glass slides, or stiff brushes.

If the sample is to be examined within 2 or 3 hours after collection, no special treatment is necessary. A periphyton sample may be maintained at 3 to 4 °C for 24 hours, but for extended storage prior to identification and enumeration, preserve as follows: To each 100 mL of water and sample, add about 3 mL 40-percent formaldehyde solution (100 percent formalin), 0.5 mL 20-percent detergent solution, and 5 to 6 drops cupric sulfate ($CuSO_4$) solution (21 g $CuSO_4$ in 100 mL distilled water). This preservative maintains cell coloration and is effective indefinitely.

Many biologists consider Lugol's solution plus acetic acid to be the best algal preservative. The solution is prepared by dissolving 10 g iodine crystals and 20 g potassium iodide in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior to use (Vollenweider, 1974). Store in an amber glass bottle. Lugol's solution is effective for at least 1 year (Weber, 1968); it facilitates sedimentation of cells and maintains fragile cell structures, such as flagella. If Lugol's solution is used as the preservative, add 1 mL of solution to each 100 mL of water that has been added to the scraped periphyton sample. Store preserved samples in the dark, preferably in amber glass bottles.

For periphyton biomass determinations, freeze the sample if ovendrying cannot be started immediately. Storage should not exceed 2 weeks.

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Young, D.W., 1945, A limnological investigation of periphyton in Douglas Lake, Michigan: Transactions of the American Microscopical Society, v. 64, p. 1-20.

# Sedgwick-Rafter method

## (B-3501-85)

**Parameter and Code:**
**Periphyton, total (cells/mm$^2$): 70945**

### 1. Applications

The method quantifies the plant (autotrophic) part of the periphyton. It is suitable for all water.

### 2. Summary of method

Samples of the periphyton community are collected, preserved, and examined microscopically for types and numbers of algae. The periphyton samples may be from natural or artificial substrates, but the dimensions of the sample area must be known.

### 3. Interferences

3.1 Suspended or deposited sediment may interfere with collection procedures and with microscopic examination.

3.2 Strong adherence of periphyton to natural and artificial substrates may result in an underestimate of cell numbers per unit area.

### 4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Artificial substrates*, glass slides, Plexiglas or polyethylene strips, tygon tubing, styrofoam, or other materials. See figures 19 and 20 for selected types of artificial substrates.

4.2 *Collecting devices*, for the removal of periphyton from natural substrates. Three devices for collecting a sample of periphyton from natural substrates are shown in figure 18.

4.3 *Microscope*, conventional light microscope, or equivalent. Bright field condensor and objectives are required, and phase contrast is desirable, particularly for taxonomic examination. A series of objectives needs to be available (10×, 20×, and 40×), and 100× oil-immersion phase-contrast objectives need to be available for examination of ultraplankton. The microscope needs to be equipped with a movable mechanical stage that has vernier scales.

4.4 *Pipet*, transfer, 1 mL, large bore.

4.5 *Sample containers*, glass or plastic, suitable for the types and sizes of samples. Sturdy plastic bags are useful containers for artificial substrates or for pieces of natural substrate.

4.6 *Scraping devices*, razor blades, stiff brushes, spatulas, or glass slides are useful for removing periphyton from artificial substrates. The edge of a glass microscope slide is excellent for scraping periphyton from hard, flat surfaces (Tilley, 1972).

4.7 *Sedgwick-Rafter counting cell*, 50×20×1 mm, and cover glass.

4.8 *Whipple disc*, placed in one ocular of the microscope.

### 5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Formaldehyde cupric sulfate solution.* Mix 1 L 40-percent aqueous formaldehyde containing 10 to 15 percent methyl alcohol with 1 mL of cupric sulfate solution.

5.5 *Lugol's solution plus acetic acid.* Dissolve 10 g iodine ($I_2$) crystals and 20 g potassium iodide (KI) in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior to use; store in an amber glass bottle (Vollenweider, 1974).

### 6. Analysis

6.1 Remove periphyton from selected substrates for a representative sample. Document the type of habitat sampled.

6.2 Adjust the scraped periphyton sample to some convenient volume of suspension, such as 50 or 100±5 mL by adding preservative solution. If used to compare community composition between bodies of water or stream reaches, habitat type and substrate should be as identical as possible.

6.3 Place the Sedgwick-Rafter counting cell on a flat surface, and place the cover glass diagonally across the cell. Thoroughly mix the sample, remove a 1-mL aliquot using a large-bore pipet, and transfer the aliquot to the Sedgwick-Rafter counting cell. As the counting cell fills, the cover glass often rotates slowly and covers the inner part of the cell, but the cover glass must not float above the rim of the cell. Allow the counting cell to stand for 15 to 20 minutes until organisms settle.

6.4 Carefully place the Sedgwick-Rafter counting cell on the mechanical stage of a calibrated microscope. Because the method assumes a homogeneous distribution of periphyton, check quickly using low power for obviously uneven distributions. If distribution appears reasonably uniform at 200× magnification, count the total number of algal cells enclosed by the Whipple disc. Consider any cell in the grid or any cell touching two intersecting borders of the grid as being

enclosed by the grid, but do not count those cells touching the opposite borders. Count and record the total number of cells in each of 20 random fields. When a 10× eyepiece and 20× objective are used, assume the total of the Whipple grid to be 0.5 mm on a side.

6.5 Some periphyton, particularly some blue-green algae, may not settle but, instead, may rise to the surface at the underside of the cover glass. When counting random fields, therefore, enumerate and record the total number of cells in the vertical column within the grid of the Whipple disc. Tabulate the number and lengths of trichomes of blue-green algae in each grid and determine the average number of cells per unit length of trichome. Consider empty diatom frustules as nonliving and do not include in calculations. Count frustules containing any part of a protoplast as having been living at the time of collection.

## 7. Calculations

7.1 Calibration factor

$$= \frac{1{,}000\ \text{mm}^2}{\text{Area of Whipple grid at 200}\times\text{ magnification (square millimeters)}}.$$

7.2 Periphyton cells per milliliter of suspended scraping

$$= \frac{\text{Total cell count} \times \text{Calibration factor}}{\text{Number of random fields} \times 1\ \text{mL}}.$$

7.3 Total periphyton cells per square millimeter of surface

$$= \frac{\text{Cells per milliliter of suspended scraping} \times \text{Total volume of scrapings (milliliters)}}{\text{Area of scraped surface (square millimeters)}}.$$

## 8. Reporting of results

Report periphyton density to two significant figures.

## 9. Precision

No numerical precision data are available.

## 10. Sources of information

Tilley, L.J., 1972, A method for rapid and reliable scraping of periphyton slides, *in* Geological Survey Research 1972: U.S. Geological Survey Professional Paper 800-D, p. D221-D222.

Vollenweider, R.A., ed., 1974, A manual on methods for measuring primary production in aquatic environments (2d ed.): Oxford and Edinburgh, Blackwell Scientific Publications, International Biological Programme Handbook 12, 225 p.

# Gravimetric method for biomass

## (B-3520-85)

**Parameters and Codes:**
**Periphyton, biomass, dry weight, total ($g/m^2$): 00573**
**Periphyton, biomass, ash weight ($g/m^2$): 00572**

Gravimetric measurements are instantaneous; that is, they measure biomass at a moment in time in a community that is constantly changing. Because of large variability in biomass within a site, and because of control of periphyton growth by numerous physical (light, current velocity, storm frequency), chemical (nutrient regime), and biological (grazing) factors, comparisons between sites are impossible using casual sampling. To be used successfully, the gravimetric method should be employed with a specific objective in mind. To make comparisons between sites, samples should be collected from environments as nearly identical as possible. Application, as a mechanism to approximate the rate of biomass accumulation (net periphyton community productivity), is more valuable than a single estimate of biomass. The latter determination generally is done by incubating clean natural or artificial substrates in as nearly identical conditions as possible, and sampling on several dates for 2 to 4 weeks, or by incubating fresh substrates for specific periods (2–4 weeks) during different seasons (Castenholz, 1960; Sladecek and Sladeckova, 1964; Lyford and Gregory, 1975; Liaw and MacCrimmon, 1978; Rodgers and others, 1979). The equal and simultaneous time periods should be reported with the data.

**1. Applications**

The method quantifies all organic mass, autotrophic and heterotrophic, living and dead, associated with the periphyton community. Gravimetric determinations are suitable for all water.

**2. Summary of method**

Samples of the periphyton community are collected from known areas of natural or artificial substrates. The dry weight and ash weight are determined.

**3. Interferences**

3.1 Inorganic matter in the sample will cause erroneously large dry and ash weights.

3.2 Dead periphyton and organic detritus that settles on the substrate will cause an overestimate of living biomass.

3.3 Natural variability generally is large for biomass and may cause a problem when the method is used for comparison.

3.4 When used as an index of production of the net periphyton community, grazing can result in an underestimate, and detrital settling will result in an overestimate of production.

3.5 Colonization rates vary depending on orientation of substrates (horizontal or vertical) because orientation affects the settling of organic and inorganic detritus. Vertical orientation is preferred because it decreases the settling problem (Castenholz, 1960; Liaw and MacCrimmon, 1978).

**4. Apparatus**

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Artificial substrates*, glass slides, Plexiglas or polyethylene strips, tygon tubing, styrofoam, or other materials. See figures 19 and 20 for selected types of artificial substrates.

4.2 *Balance*, capable of weighing to at least 0.1 mg.

4.3 *Collecting devices*, for the removal of periphyton from natural substrates. Three devices for collecting a sample of periphyton from natural substrates are shown in figure 18.

4.4 *Desiccator*, containing silica gel or anhydrous calcium sulfate.

4.5 *Drying oven*, thermostatically controlled for use at 105 °C.

4.6 *Filtration apparatus*, non-metallic, and has a vacuum.

4.7 *Forceps*, stainless steel, smooth tip, or tongs.

4.8 *Glass filters*, 47-mm diameter disks.

4.9 *Muffle furnace*, for use at 500 °C.

4.10 *Porcelain crucibles.*

4.11 *Sample containers*, glass or plastic, suitable for the types and sizes of samples. Sturdy plastic bags are useful containers for artificial substrates or for pieces of natural substrate. Do not use glass containers for samples to be frozen.

4.12 *Scraping devices*, razor blades, stiff brushes, spatulas, or glass slides are useful for removing periphyton from artificial substrates. The edge of a glass microscope slide is excellent for scraping periphyton from hard, flat surfaces (Tilley, 1972).

**5. Reagents**

5.1 *Distilled or deionized water.*

**6. Analysis**

6.1 Calculate the tare weight of a crucible containing a glass-fiber filter. Heat at 500 °C for about 20 minutes, cool

to room temperature in a desiccator, and weigh to the nearest 0.1 mg.

6.2 Filter the water and the scrapings from the periphyton strip in the sample bottle through the tared glass-fiber filter. Place filter in crucible and dry at 105 °C to a constant weight. Cool crucibles containing dried periphyton to room temperature in a desiccator before weighing. Weigh as rapidly as possible to decrease moisture uptake by the dried residue. Use these weight values to calculate dry weight.

6.3 Place the crucible containing the dried residue in a muffle furnace at 500 °C for 1 to 4 hours. Cool to room temperature.

6.4 Moisten the periphyton ash using distilled water and again ovendry at 105 °C to constant weight as described in 6.2. Use these weight values to calculate ash weight.

## 7. Calculations

7.1 Dry weight of periphyton (grams per square meter)

$$= \frac{\text{Dry weight of crucible and residue (grams)} - \text{Tare weight of crucible (grams)}}{\text{Area of scraped surface (square meters)}} .$$

7.2 Ash weight of periphyton (grams per square meter)

$$= \frac{\text{Ash weight of crucible and residue (grams)} - \text{Tare weight of crucible (grams)}}{\text{Area of scraped surface (square meters)}} .$$

## 8. Reporting of results

Report periphyton biomass to three significant figures.

## 9. Precision

No numerical precision data are available.

## 10. Sources of information

Castenholz, R.W., 1960, Seasonal changes in the attached algae of freshwater and saline lakes in the Lower Grand Coulee, Washington: Limnology and Oceanography, v. 5, no. 1, p. 1-28.

Liaw, W.K., and MacCrimmon, H.R., 1978, Assessing changes in biomass of riverbed periphyton: Internationale Revue der Gestamten Hydrobiolie, v. 63, no. 2, p. 155-171.

Lyford, J.H., and Gregory, S.V., 1975, The dynamics and structure of periphyton communities in three Cascade Mountain streams: Verhandlung Internationale Vereinigung Limnologie, v. 19, p. 1610-1616.

Rodgers, J.H., Jr., Dickson, K.L., and Cairns, John, Jr., 1979, A review and analysis of some methods used to measure functional aspects of periphyton, *in* Weitzel, R.L., ed., Methods and measurements of periphyton communities—A review: American Society for Testing and Materials Special Technical Publication 690, p. 142-167.

Sladecek, Vladimir, and Sladeckova, Alena, 1964, Determination of periphyton production by means of the glass slide method: Hydrobiologia, v. 23, no. 1, p. 125-158.

Tilley, L.J., 1972, A method for rapid and reliable scraping of periphyton slides, *in* Geological Survey Research 1972: U.S.Geological Survey Professional Paper 800-D, p. D221-D222.

# Permanent-slide method for periphytic diatoms

## (B-3540-85)

**Parameter and Code: Not applicable**

This procedure enables preparation of permanent mounts using a minimum of time and equipment. Numerous alternative methods for clearing diatom frustules (cell walls) and mounting exist in the literature. Alternative methods for clearing include nitric acid digestion of tissue on the slide (Knudsen, 1966), sulfuric acid and potassium permanganate (Hasle and Fryxell, 1970), hydrochloric acid (HCl) (Cupp, 1943), and potassium permanganate and HCl (Hasle, 1978). Hydrogen peroxide and potassium permanganate (Van der Werff, 1953), hydrogen peroxide and ultraviolet light (Swift, 1967), and hydrogen peroxide after mild heating (Wong, 1975) also have been used for tissue digestion. The reader is referred to the original papers for the details of these procedures.

**1. Applications**

This qualitative method is suitable for all water. Advantages of the method are that a permanent mount is prepared, and clearing of the cells enhances observation of frustule detail. The method, therefore, is important in the taxonomic study of diatoms.

**2. Summary of method**

The diatoms in a sample are concentrated, the cells are cleared, and a permanent mount is prepared. The mount is examined microscopically, and the number of diatom taxa is calculated from strip counts.

**3. Interferences**

3.1 Inorganic particulate matter, including salt crystals, interferes with mount preparation but can be decreased by sample washing.

3.2 The method does not distinguish living from dead diatoms. At certain seasons, particularly during low flow, more than one-half the cells may be dead (Pryfogle and Lowe, 1979). As a result, permanent mounts may provide an inaccurate estimate of community composition.

**4. Apparatus**

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Artificial substrates*, glass slides, Plexiglas or polyethylene strips, tygon tubing, styrofoam, or other materials. See figures 19 and 20 for selected types of artificial substrates.

4.2 *Balance*, that has an automatic tare.

4.3 *Centrifuge*, either swing-out type or fixed-head cup type, 3,000 to 4,000 r/min, 15- to 50-mL conical 100-mL pear-shaped *centrifuge tubes*, and simple *siphoning* or *suction device* to remove excess fluid after centrifugation.

4.4 *Collecting devices*, for the removal of periphyton from natural substrates. Three devices for collecting a sample of periphyton from natural substrates are shown in figure 18.

4.5 *Cover glasses*, 18×18 or 22×22 mm, No. 1½, and *microscope slides*, glass, 76×25 mm.

4.6 *Forceps*, curved tip.

4.7 *Graduated cylinders*, plastic, of sufficient capacity (100 and 500 mL and 1 L are convenient sizes) for measuring known volumes of water samples.

4.8 *Hotplate*, thermostatically controlled to 538 °C. It is convenient to have a second hotplate for operation at about 93 to 121 °C as described in 6.10.

4.9 *Microscope*, conventional light microscope, or equivalent. Bright field condensor and objectives are required, and phase contrast is desirable, particularly for taxonomic examination. A series of objectives needs to be available (10×, 20×, and 40×), and 100× phase-contrast oil-immersion objectives need to be available for examination of ultraplankton. The microscope needs to be equipped with a movable mechanical stage that has vernier scales.

4.10 *Pipets*, 1-mL or 10-mL capacity, sterile.

4.11 *Sample containers*, glass or plastic, suitable for the types and sizes of samples. Sturdy plastic bags are useful containers for artificial substrates or for pieces of natural substrates.

4.12 *Scraping devices*, razor blades, stiff brushes, spatulas, or glass slides are useful for removing periphyton from artificial substrates. The edge of a glass microscope slide is excellent for scraping periphyton from hard, flat surfaces (Tilley, 1972).

4.13 *Whipple disc*, placed in one ocular of the microscope.

**5. Reagents**

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Formaldehyde cupric sulfate solution*. Mix 1 L 40-percent aqueous formaldehyde containing 10 to 15 percent methyl alcohol with 1 mL cupric sulfate solution.

5.5 *Immersion oil*, Cargille's nondrying type A.

5.6 *Lugol's solution plus acetic acid.* Dissolve 10 g iodine ($I_2$) crystals and 20 g potassium iodide (KI) in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior

to use; store in an amber glass bottle (Vollenweider, 1974).

5.7 *Mounting medium* (table 13). Generally, mounting media should have a refractive index different than that of diatom frustules. Diatom frustules have a refractive index of approximately 1.15 (Reid, 1978).

**6. Analysis**

6.1 Remove the periphyton from the substrate using a suitable device.

6.2 By vigorous shaking, thoroughly disperse the periphyton in about 100 mL of preservative, or distilled water, if working with unpreserved material.

6.3 If the sample contains great numbers of periphyton, as typically occurs in eutrophic water, dilute the sample. To dilute, thoroughly mix 50 mL sample with 50 mL distilled water (1:1 dilution) and proceed to 6.4. If microscopic examination reveals a concentration of periphyton still too numerous to count, thoroughly mix 50 mL 1:1 dilution with 50 mL distilled water (1:4 dilution). Make additional dilutions as appropriate.

6.4 If concentration is necessary, allow the sample to settle undisturbed in the sample container for 4 hours per centimeter of depth to be settled. After settling, weigh the sample container on an automatic tare balance.

6.5 Carefully siphon the supernatant to avoid disturbance of the settled material. Place sample container and remaining sample on balance and weigh. The decrease in weight (in grams) is equivalent to the number of milliliters of supernatant removed. Use the same method to obtain the volume of concentrate.

6.6 If the sample was collected from seawater or saline lakes, wash the periphyton, using distilled water, at least three times to ensure that the permanent mounts are not obscured by salt crystals. Add about 10 mL distilled water to the concentrate in the centrifuge tube, gently shake the tube to suspend the residue, fill the tube with distilled water, and centrifuge for 20 minutes. Decant the supernatant fluid and repeat the washing process two more times.

6.7 Place two or three drops of the concentrate on each of three or four cover glasses.

6.8 With the concentrate side up, place the cover glass on a hotplate and heat, slowly at first to prevent splattering, to about 538 °C (a higher temperature will melt diatom valves) for 30 minutes.

6.9 Remove cover glass from the hotplate and cool.

6.10 Place a drop of mounting medium (table 13) on a microscope slide and heat at about 93 to 121 °C for 3 to 4 minutes.

6.11 Invert the cover glass, concentrate side down, on the heated medium. Apply slight pressure to the cover glass (for example, with a pencil eraser) until visible air bubbles disappear. Remove slide from hotplate and allow to cool. If bubbles still are present under the cover glass, heat the slide and gently apply additional pressure to the cover glass. Label the slide to identify sample.

6.12 Examine the slide using the 100× objective (oil immersion). Count and identify all diatom taxa found in several lateral strips the width of the Whipple disc. Identify and tabulate 200 to 300 diatom cells, if possible. Generally, at least 100 individuals of the most common species should be enumerated. Ignore frustule fragments. Thin-walled forms, such as *Rhizosolenia eriensis* and *Melosira crenulata*, may be difficult to observe when using this method (Weber, 1966, p. 3). If a microscope that has a mechanical stage is used, recording of the $x$ and $y$ coordinates of lateral strips or individual cells enables investigators to later recheck and verify identification (Wong, 1975).

**7. Calculations**

Percent occurrence of each species

$$= \frac{\text{Number of diatoms of a given species}}{\text{Total number of diatoms tabulated}} \times 100.$$

**8. Reporting of results**

Report percentage composition of diatoms to the nearest whole number. Report taxa and number of organisms per taxa.

**9. Precision**

No numerical precision data are available.

**10. Sources of information**

Cupp, E.E., 1943, Marine plankton diatoms of the west coast of North America: Bulletin of the Scripps Institute of Oceanography, University of California at La Jolla, v. 5, p. 1-238.

Hasle, G.R., 1978, Diatoms, *in* Sournia, Alain, ed., Phytoplankton manual: Paris, UNESCO, Monographs on Oceanographic Methodology 6, p. 136-142.

Hasle, G.R., and Fryxell, G., 1970, Diatoms—Cleaning and mounting for light and electron microscopy: Transactions of the American Microscopical Society, v. 89, p. 469-474.

Knudsen, J., 1966, Biological techniques: New York, Harper and Row, 525 p.

Pryfogle, P.A., and Lowe, R.L., 1979, Sampling and interpretation of epilithic lotic diatom communities, *in* Weitzel, R.L., ed., Methods and measurements of periphyton communities—A review: American Society for Testing and Materials Special Technical Publication 690, p. 77-89.

Reid, F.M., 1978, Permanent slides, *in* Sournia, Alain, ed., Phytoplankton manual: Paris, UNESCO, Monographs on Oceanographic Methodology 6, p. 115-118.

Swift, Elijan, 1967, Cleaning diatom frustules with ultraviolet radiation and peroxide: Phycologia, v. 6, p. 161-163.

Tilley, L.J., 1972, A method for rapid and reliable scraping of periphyton slides, *in* Geological Survey Research 1972: U.S.Geological Survey Professional Paper 800-D, p. D221-D222.

Vollenweider, R.A., ed., 1974, A manual on methods for measuring primary production in aquatic environments (2d ed.): Oxford and Edinburgh, Blackwell Scientific Publications, International Biological Programme Handbook 12, 225 p.

Van der Werff, A., 1953, A new method for concentrating and cleaning diatoms and other organisms: International Association Theoretical and Applied Limnology Proceedings, v. 1, p. 276-277.

Weber, C.I., 1966, A guide to the common diatoms at water pollution surveillance system stations: Cincinnati, Ohio, Federal Water Pollution Control Administration, Water Pollution Surveillance, 98 p.

Wong, R.L., 1975, Diatom flora of the phytoplankton of San Francisco Bay: San Francisco, San Francisco State University, M.S. thesis, 144 p.

# Inverted-microscope method for the identification and enumeration of periphytic diatoms

## (B-3545-85)

**Parameter and Code:**
**Diatoms, total, periphyton (number/mm$^2$): 81804**

### 1. Applications

The method is suitable for all water. The diatoms are cleared, making identification of species possible. Reliable quantitative enumeration is possible after the diatoms are separated from one another and from extracellular organic matter.

### 2. Summary of method

Periphytic diatoms are collected by scraping them from their substrate. Organic components, including gelatinous stalks and matrices and cellular components in the diatoms, are decomposed by oxidation. The diatoms in a sample are concentrated, and a permanent mount is prepared from a 0.1-mL aliquot. The mount is examined microscopically for the purpose of identification and tabulation, and the cleared diatoms are placed in a counting cell for enumeration.

### 3. Interferences

Large quantities of sediment associated with the periphyton may obscure the diatoms in the counting cell. Sediment and other particulate matter, including salt crystals and carbonaceous residues, interfere with slide-mount preparation.

### 4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Artificial substrates*, glass slides, Plexiglas or polyethylene strips, tygon tubing, styrofoam, or other materials. See figures 19 and 20 for selected types of artificial substrates.

4.2 *Collecting devices* for the removal of periphyton from natural substrates. Three devices for collecting a sample of periphyton from natural substrates are shown in figure 18.

4.3 *Counting cell*, 26×76-mm glass slide that has 12-mm circular hole, covered by cementing No. 1½ cover glass to slide, and No. 1½ cover glass for top of cell.

4.4 *Cover glasses*, 18×18 or 22×22 mm, No. 1½, and *microscope slides*, glass, 76×25 mm.

4.5 *Graduated cylinders*, plastic, of sufficient capacity (100 and 500 mL and 1 L are convenient sizes) for measuring known volumes of water samples.

4.6 *Hotplate*, thermostatically controlled for operation at about 93 to 121 °C.

4.7 *Inverted microscope.*

4.8 *Microspatula*, 0.1 g.

4.9 *Sample containers*, glass or plastic, suitable for the types and sizes of samples. Sturdy plastic bags are useful containers for artificial substrates or for pieces of natural substrates.

4.10 *Scraping devices*, razor blades, stiff brushes, spatulas, or glass slides are useful for removing periphyton from artificial substrates. The edge of a glass microscope slide is excellent for scraping periphyton from hard, flat surfaces (Tilley, 1972).

4.11 *Vial*, 10 mL, glass, disposable (for reference sample).

4.12 *Water aspirator.*

### 5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Formaldehyde cupric sulfate solution.* Mix 1 L 40-percent aqueous formaldehyde containing 10 to 15 percent methyl alcohol with 1 mL of cupric sulfate solution.

5.5 *Hydrogen peroxide* ($H_2O_2$), 30 percent.

5.6 *Immersion oil*, Cargille's nondrying type A.

5.7 *Lugol's solution plus acetic acid.* Dissolve 10 g iodine ($I_2$) crystals and 20 g potassium iodide (KI) in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior to use; store in an amber glass bottle (Vollenweider, 1974).

5.8 *Mounting medium* (table 13). Generally, mounting media should have a refractive index different than that of diatom frustules. Diatom frustules have a refractive index of approximately 1.15 (Reid, 1978).

5.9 *Potassium dichromate* ($K_2Cr_2O_7$) or *ammonium persulfate* [$(NH_4)_2S_2O_8$].

### 6. Analysis

6.1 Place the scraped periphyton sample in a graduated cylinder (100–500 mL).

6.2 If formaldehyde solution perservatives have been added, wash (Note 1) the sample by filling the cylinder, to capacity, with distilled water and allow the periphyton to settle at a minimum rate of 2 hours per centimeter of depth. Although centrifugation accelerates sedimentation, it may damage fragile diatoms and, therefore, is not recommended. To determine when settling is complete, periodically examine the supernatant microscopically using the inverted micro-

scope and the counting cells. When settling is completed, aspirate all but 5 to 10 percent of the supernatant, being careful not to disturb the sedimented material. Repeat the entire procedure several times.

Note 1: The washing procedure is important because samples concentrated for diatom analysis commonly contain dissolved materials, such as salts, preservatives, and detergents, that will leave interfering residues on a permanent-slide mount. Certain preservatives, such as formaldehyde solution, will produce extremely exothermic reactions when hydrogen peroxide is added.

6.3 To the rinsed, concentrated sample, add hydrogen peroxide in a volume approximately five times the concentrate volume and allow the sample to stand for 7 days. Ultraviolet radiation is an effective catalyst for hastening the oxidation process. Do not proceed to step 6.5 until all hydrogen peroxide has been reduced, as evidenced by the cessation of bubble formation.

6.4 If large quantities of extracellular organic matter are present, add a microspatula (approximately 0.1 g) of potassium dichromate (or ammonium persulfate) to the mixture inside a fume hood. This will initiate an exothermic reaction. After the reaction is completed (5–10 minutes), the potassium dichromate solution will change from purple to gold.

6.5 Fill the graduated cylinder with distilled water. Allow the mixture to stand for a minimum of 2 hours per centimeter of depth so that the cleared periphyton will settle to the bottom. Aspirate the mixture, carefully removing and discarding the liquid without disturbing the sediment on the bottom of the cylinder. Repeat this procedure until the supernatant is colorless.

6.6 Mix the concentrated sample well (but not vigorously), and place a small quantity onto each of three cover glasses and spread.

6.7 Place the cover glasses, concentrate side up, on a warm hotplate to increase the evaporation rate, but not enough to boil. Evaporate to dryness.

6.8 Using a glass rod, place several drops of mounting medium, diluted according to manufacturer's instructions, in the center of the cover glass. Commercially available mounting medium (table 13) ensures easily handled permanent mounts for examination during oil immersion. Medium that has high index of refraction (1.65+) is best for mounting diatoms. The greater the index of refraction, the greater the contrast of the microscopic image. Diatoms have a refractive index of about 1.15 and are invisible in medium of similar index.

6.9 Heat the cover glasses slowly, increasing the temperature until all the diluting solvent has been evaporated from the mounting medium. Cool and place the cover glass (concentrate side down) on the center of the slide, and reheat slowly until the medium has flowed to the edges of the cover glass. Remove from source of heat and cool. Ring the cover glass for permanence, if desired.

6.10 Examine the slides at 1,000× magnification (oil immersion) using a compound binocular microscope, and identify the diatom taxa.

6.11 If sediment does not interfere with the identification, adjust the volume of the concentrate in step 6.5 to obtain a frustule count of 5 to 10 frustules per field. Record this adjusted volume as the total (or final) volume. Mix the sample concentrate well (but not vigorously), and pipet sample into each of 10 counting cells. Slide cover glasses into place immediately.

6.12 Place the counting cell on the mechanical stage of a calibrated inverted microscope. Count and identify the diatoms in at least 50 randomly chosen fields at 300 to 500× magnification. Count a minimum of 100 diatom frustules, 300 to 500 if possible, distributing the count among cells using five fields per cell (Woelkerling and others, 1976). If broken or separated frustules are observed, count full half frustules (complete valves) and tabulate accordingly. If taxa that are not on the compiled taxa list are observed, identify them at 800 to 1,000× magnification.

## 7. Calculations

7.1 Diatoms per milliliter of suspended scraping

$$= \frac{\text{Total count}}{\text{Number of fields} \times \text{Chamber depth (centimeters)} \times \text{Field area (square centimeters)}}.$$

7.2 Total diatoms per square millimeter of surface

$$= \frac{\text{Diatoms per milliliter of suspended scraping} \times \text{Total volume of scraping (milliliters)}}{\text{Area of scraped surface (square millimeters)}}.$$

7.3 Percent occurrence of each species

$$= \frac{\text{Number of diatoms of a given species}}{\text{Total number of diatoms tabulated}} \times 100.$$

## 8. Reporting of results

Report diatom counts to two significant figures.

## 9. Precision

No precision data are available.

## 10. Sources of information

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# MACROPHYTES

## Introduction

Macrophytes include vascular plants, bryophytes, and algae that can be seen without magnification. The aquatic macrophytes referenced in this text are nonwoody macrophytes commonly found in wetlands or deep-water habitats (Cowardin and others, 1979). The characteristic vascular plant forms found in aquatic habitats are: (1) Emergent rooted aquatics, (2) floating-leaved rooted aquatics, (3) submersed rooted aquatics, and (4) free-floating aquatics. Some of these plants may form marginal mats or floating islands.

Bryophytes, the mosses and liverworts, generally are less conspicuous than the vascular plants. In swiftly flowing water, they generally grow attached to submerged or partly submerged rocks. In quiet water, mosses and liverworts may be attached to submerged rocks and mud substrata alone or may be among rooted vascular plants.

Algae are plants that lack true roots, stems, and leaves. They include the smallest of chlorophyll-bearing plants that consist of a single cell (commonly found in the phytoplankton or periphyton) as well as marine representatives ranging to several tens of meters in length. Freshwater species of algae occur as individual plants, colonies, or patches attached to rocks in flowing water. Such plants may be gray, green, blue-green, or olive, and may be slimy to the touch, such as *Batrachospermum*; or, they may be green and have a coarse filamentous structure and profuse lateral branching, such as *Cladophora*. In slow flowing or quiet water, algae that have stemlike and leaflike structures frequently are found. These plants commonly have a glistening or translucent appearance (*Nitella*), or they may be encrusted with lime, which gives rise to the common name stonewort (*Chara*). All of these types of algae also may be found in brackish coastal water or saline inland water.

Distribution and growth of aquatic macrophytes depend on depth of water, illumination, nutrient availability, water quality, substrate, and water velocity. Sometimes the rooted vascular plants are arranged in zones corresponding to successively greater water depths. The predominant vegetation in each deeper zone is composed of species more tolerant of water depth or decreasing illumination. These zones may be greatly compressed in turbid water. The processes of erosion and deposition and the resultant substrate composition partially control the extent to which plant zones develop. Free-floating aquatic plants may occur anywhere on the water surface; their distribution is controlled by water velocity and wind. The growth of aquatic macrophytes is related to the availability of nutrients. In some bodies of water, nutrient enrichment results in excessive growth of macrophytes that may become a major nuisance and may constitute an important water-quality problem. However, long-term nutrient enrichment may alter the macrophyte-phytoplankton-nutrient balance and may produce changes in species composition or a decline in populations of aquatic macrophytes (Haslam, 1978). Tissue analysis of plants may provide information for evaluating nutrient supplies in natural water (Gerloff and Krombholz, 1966), for determining nutrient requirements for particular plant species (Fitzgerald, 1969), or for studying bioaccumulation of trace metals (Mayes and others, 1977).

## Collection

Samples of macrophytes are collected by hand or with grappling hooks, rakes, oyster tongs, or dredges. Entire plants should be collected, including flowers and seeds, if present, and roots and rhizomes or tubers, if possible. During floral surveys, all habitats should be sampled in an effort to collect common and rare species. For some investigations, the relative abundance of plant species in the study area should be noted. For quantitative studies, a uniform sampling system for plant collection should be devised to provide some measure of abundance and productivity.

Plants to be placed in a herbarium or preserved for identification or further study should be pressed and mounted using standard techniques. Place emergent rooted aquatics and free-floating or floating-leaved rooted aquatics that have large coarse leaves (*Nymphaea*, or *Pistia*, for example) in a plant press for preservation. Use paper toweling or other absorbent material to soak up as much moisture from the specimens as possible before preparing them for the press. Carefully arrange each plant on one-half of a folded sheet of newspaper. Bend stems and leaves where necessary, but keep the plants as flat and as widely spread as possible. Label each plant for location collected, date collected, and species, if known. Fold the other one-half of the newspaper over each flattened plant, sandwich between two botanical driers, and place in a plant press. Many sheets that contain specimens may be added to the press, but each preparation must be separated by a botanical drier. Tie or strap the press securely.

Replace the damp botanical driers frequently (daily or weekly, depending on water content of plant material) until all plant parts are completely dry. This replacement is necessary if plant specimens are to be preserved satisfactorily. Plants being pressed should be kept cool to help control spoilage of the wet material, unless the press containing the plants is placed in a botanical drying rack to hasten drying using artificial heat. Before proceeding with the heat method of drying macrophytes, read the techniques described by Lawrence (1960, p. 241-243).

Submersed rooted aquatics, especially those with fine strap-like or dissected leaves, are limp and fragile and should not be handled in air. The same is true for algae. Wash thoroughly to remove epiphytes and debris, and float the specimen in water in a flat tray or sink. Arrange plant, slip mounting sheet under it, and remove from water, or drain water and allow plant to settle on paper. Good-quality herbarium paper can be used, or the plant can be floated on other paper and subsequently mounted on a herbarium sheet. For species that have emergent flowers (for example, *Utricularia*), remove flowering parts prior to floating and press separately by standard method. Place paper and plant on one-half of a folded sheet of newspaper and place a sheet of waxed paper directly on top of plant material. Fold the other one-half of the newspaper over the plant, sandwich between two botanical driers, and place in a plant press. Use of a drying rack and artificial heat is recommended.

Duckweed (*Lemnaceae*) should be floated onto index cards and placed between newspaper sheets in the plant press. The upper and lower sides of these plants should be visible when arranged on the index cards. When dry, the specimens will fall off the card and should be placed in a packet or mounted on a herbarium sheet.

After drying, glue or cloth tape should be used to affix specimens to herbarium paper. Packets of flowers, seeds, or small, delicate specimens should be mounted on the sheet with the remainder of the plant. Many algae have a natural muscilagenous coating that serves as a natural glue when dried.

Preserve small specimens of vascular plants and bryophytes in 70-percent ethyl alcohol, 2-percent solution of formalin, 2-percent oxyquinoline, or 8-hydroxyquinoline sulfate solution. Add a volume of preservative at least equal to the volume of plant material to ensure adequate preservation. Although this preservation is adequate for macrophytes in general, freshwater algae should be preserved as follows: to each 100 mL of sample, add about 3 mL 100-percent formalin (37- to 40-percent formaldehyde solution), 0.5 mL 20-percent detergent solution, and 5 to 6 drops cupric sulfate solution. For marine or brackish-water algae, use 4- to 5-percent final formalin solution made with the water in which the plant was collected. For large marine species, for example, *Laminaria*, use a mixture containing 10 percent phenol, 30 percent alcohol, 30 percent glycerine, and 30 percent water (Taylor, 1957). This will maintain flexibility and prevent specimens from becoming brittle.

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# Floral survey (qualitative method)

## (B-4501-85)

**Parameter and Code: Not applicable**

**1. Applications**

The method is suitable for all water.

**2. Summary of method**

Specimens from each habitat are collected and identified using appropriate references and taxonomic keys. Specimens are preserved or pressed and mounted for herbarium collection or further study.

**3. Interferences**

Missing or incompletely developed plant parts (flowers, seeds, or other parts) or improperly preserved plant material may make identification of a specimen difficult.

**4. Apparatus**

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Botanical driers.* These driers are absorbent pads, measuring approximately 30×46 cm, for use in plant presses. When preserving submersed aquatics, artificial heat is needed with driers.

4.2 *Collecting equipment*, appropriate to the objectives of the study, the type of substrate, and the depth of water. Examples of suitable equipment are:

4.2.1 *Dredge.*

4.2.2 *Oyster tongs*, that have steel blades welded across teeth and small cord attached across opening to control size of sample (Sincock and others, 1965; Kerwin and others, 1976; Carter and Haramis, 1980).

4.2.3 *Plant grappling hook.* A simple grappling hook may be fabricated by binding the shanks of several hooks from wire coathangers together using light-weight wire. Make a loop on an extra-long shank for attaching a line.

4.2.4 *Steel garden rake.*

4.3 *Microscope*, binocular, wide-field, dissecting-type, and fluorescent lamp.

4.4 *Newspaper stock*, folded to about 29×42 cm.

4.5 *Plant press.*

4.6 *Sample containers*, wide-mouth glass or plastic jars and leakproof caps or sealable plastic bags.

**5. Reagents**

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Ethyl alcohol*, 70 percent.

5.5 *Formaldehyde solution*, 37 to 40 percent (formalin, 100 percent).

5.6 *Oxyquinoline or 8-hydroxyquinoline sulfate*, 2 percent. Dissolve 2 g 8-hydroxyquinoline sulfate in 50 mL distilled water and dilute to 100 mL. This preservative is used as a general substitute for either alcohol or formaldehyde solution for preserving macrophytes (Swingle, 1930; Lawrence, 1960, p. 255). This preservative lacks most of the objectionable features of formaldehyde solution and particularly is useful onsite because small envelopes or capsules of measured quantities of powder may be mixed with water as needed (Moore, 1950).

**6. Analysis**

Identify plant specimens using an appropriate taxonomic key, such as Muenscher (1944), Smith (1950), Conrad (1956), Wood (1967), Radford and others (1968), Fassett (1969), Britton and Brown (1970), Fernald (1970), Hotchkiss (1972), Correll and Correll (1975), and Beal (1977). A stereoscopic microscope may be required.

**7. Calculations**

None required.

**8. Reporting of results**

List the taxa of macrophytes identified.

**9. Precision**

No numerical precision data are available.

**10. Sources of information**


Beal, E.D., 1977, A manual of marsh and aquatic vascular plants of North Carolina with habitat data: North Carolina Agricultural Experiment Station Technical Bulletin 247, 298 p.

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# Distribution and abundance (quantitative method)

## (B-4520-85)

**Parameter and Code:**
**Macrophytes, total (number/$m^2$): 70944**

**1. Applications**

The method is suitable for all water.

**2. Summary of method**

The distribution of macrophytes is determined onsite and plotted on a map of the study area. The size of the subareas inhabited by different kinds of macrophytes or the size of the vegetated area can be determined by planimetry or dot grid if desired. Transect, grid, or quadrat sampling schemes are developed, and floral composition and relative abundance (percent cover, density, frequency of occurrence) are established.

**3. Interferences**

Physical factors, such as depth of water, may interfere with determination of macrophyte distribution and abundance. Missing or incompletely developed plant parts (flowers, seeds, or other parts) or improperly preserved plant material may make identification of a specimen difficult.

**4. Apparatus**

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Aerial photographs*, at appropriate scales. Color infrared photographs are best for emergent rooted, floating-leaved rooted, or free-floating aquatic macrophytes; natural color or black-and-white photographs are preferred for submersed rooted aquatic macrophytes (Carter, 1977). Existing photographs can be obtained by contacting the National Cartographic Information Center (NCIC) in Reston, Va., or the EROS Data Center (EDC) in Sioux Falls, S. Dak.

4.2 *Base map*, at appropriate scale. Scale-stable base maps may be obtained from the Water Resources Division Publications Office at standard scales (for example, 1:24,000, 1:250,000, 1:1,000,000).

4.3 *Botanical driers*. These driers are absorbent pads, measuring approximately 30×46 cm, for use in plant presses. When preserving submersed aquatics, artificial heat is needed with driers.

4.4 *Collecting equipment*, appropriate to the objectives of the study, the type of substrate, and the depth of water. Examples of suitable equipment are:

4.4.1 *Dredge.*

4.4.2 *Oyster tongs*, that have steel blades welded across teeth and small cord attached across opening to control size of sample (Sincock and others, 1965; Kerwin and others, 1976; Carter and Haramis, 1980).

4.4.3 *Plant grappling hook.* A simple grappling hook may be fabricated by binding the shanks of several hooks from wire coathangers together using light-weight wire. Make a loop on an extra-long shank for attaching a line.

4.4.4 *Steel garden rake.*

4.5 *Microscope*, binocular, wide-field, dissecting-type, and fluorescent lamp.

4.6 *Newspaper stock*, folded to about 29×42 cm.

4.7 *Plant press.*

4.8 *Polar planimeter*, or dot grid at appropriate scale.

4.9 *Sample containers*, wide-mouth glass or plastic jars and leakproof caps or sealable plastic bags.

4.10 *Surveying or other equipment*, suitable for developing transect, grid, and quadrat sampling schemes (Cox, 1967; Mueller-Dombois and Ellenberg, 1974).

**5. Reagents**

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Ethyl alcohol*, 70 percent.

5.5 *Formaldehyde solution*, 37 to 40 percent (formalin, 100 percent).

5.6 *Oxyquinoline or 8-hydroxyquinoline sulfate*, 2 percent. Dissolve 2 g 8-hydroxyquinoline sulfate in 50 mL distilled water and dilute to 100 mL. This preservative is recommended as a general substitute for either alcohol or formaldehyde solution for preserving macrophytes (Swingle, 1930; Lawrence, 1960, p. 255). This preservative lacks most of the objectionable features of formaldehyde solution and particularly is useful onsite because small envelopes or capsules of measured quantity of powder may be mixed with water as needed (Moore, 1950).

**6. Analysis**

6.1 Identify plant specimens using an appropriate taxonomic key, such as Muenscher (1944), Smith (1950), Conrad (1956), Wood (1967), Radford and others (1968), Fassett

(1969), Britton and Brown (1970), Fernald (1970), Hotchkiss (1972), Correll and Correll (1975), and Beal (1977). A stereoscopic microscope may be required.

6.2 Determine the mappable units (discrete vegetative communities, associations, or homogeneous stands) and choose the appropriate scale for mapping (Kuchler, 1967). This determination should be made after onsite observations and identification of mappable units using aerial photographs when available.

6.3 Determine the major floristic components of each map unit by onsite observation and sampling. If abundance is included, calculate percent cover, density, or frequency of occurrence, or all three, from transect or quadrat samples (Cox, 1967; Mueller-Dombois and Ellenberg, 1974).

6.4 Outline map units on map base or overlay material. Map legend or explanation should clearly identify each map unit and its symbol or color. Map also should include a scale and north arrow or latitude-longitude tick marks.

6.5 Determine the area (in square meters or square kilometers) covered by each vegetative community, association, or homogeneous stand, using a polar planimeter or dot grid.

## 7. Calculations

7.1 Percent cover

$$= \frac{\text{Area covered by community, association, or homogeneous stand (square meters or square kilometers)}}{\text{Total area (square meters or square kilometers)}} \times 100.$$

7.2 Density

$$= \frac{\text{Number of individual plants}}{\text{Area sampled (square meters or square kilometers)}}.$$

7.3 Frequency of occurrence

$$= \frac{\text{Number of plots in which species occurs}}{\text{Total number of plots sampled}}.$$

## 8. Reporting of results

8.1 List the taxa of macrophytes identified.

8.2 The map shows distribution. Report the percent cover, density, or frequency of occurrence for each community, association, or homogeneous stand.

## 9. Precision

No numerical precision data are available.

## 10. Sources of information


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Wood, R.D., 1967, Charophytes of North America: West Kingston, R.I., Stellas' Printing, 72 p.