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Standard Test Method for Analysis of Phytoplankton in Surface Water by the Sedgwick-Rafter Method[1]

This standard is issued under the fixed designation D 4148; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers determining the density and taxonomic classification of phytoplankton. It is applicable both to relatively sparse or dense phytoplankton concentrations, provided the suspended-sediment concentration is low. The Sedgwick Rafter (S-R) method requires less costly apparatus than does the inverted microscope method but gives less accurate results. The inherent inaccuracy in the Sedgwick-Rafter method is due to the design of the counting chamber and cannot be circumvented by a different choice of optics. For this reason, the S-R method is limited to the use of objective lenses having a working distance of approximately 1.6 mm or more. With 10× oculars the maximum overall magnification is approximately 250×. High concentrations of suspended sediment can obscure the algal cells, and thus cause interference.

1.2 This test method is applicable to both freshwater and marine samples.

1.3 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific precautionary information see Section 8.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water
D 3370 Practices for Sampling Water
D 4149 Classification for Sampling Phytoplankton in Surface Waters

2.2 Various taxonomic keys are required for identification of the algae. No single key is suitable for all species likely to be encountered. (See Greeson 1977; Weber 1973.)

3. Summary of Test Method

3.1 The microscope is calibrated to determine the field size on the superimposed ocular grid. A Sedgwick-Rafter chamber is filled with a preserved phytoplankton sample. After the algae settles to the bottom, the chamber is examined microscopically at 200 to 250× for the presence of algae. Those algal cells lying within the border of the ocular grid are identified and enumerated. The tally is used to calculate the algal density in cells per millilitre.

4. Significance and Use

4.1 Phytoplankton are basic to the food chain in all aquatic environments. In addition, they have long been considered to be important indicators of water-quality conditions. Phytoplankton data are also frequently used in the planning and design of water-treatment facilities and reservoirs.

5. Interferences

5.1 The presence of suspended sediment may obscure algal cells, making identification difficult. Colonial forms and the occurrence of algae in trichomes make the estimation of cell numbers difficult. Some preservation techniques may cause a loss of flagella, hampering identification.

6. Apparatus

6.1 *Microscope*, compound, with 10× oculars and 10×, 25×, 40×, and 90× objectives, substage condenser, and mechanical stage.

6.2 *Ocular Micrometer*, with Whipple grid.

6.3 *Sedgwick-Rafter Counting Cell*, 50 by 20 by 1 mm.

6.4 *Stage Micrometer*.

6.5 *Transfer Pipet*, 1-mL.

6.6 *Microscope Slides and Cover Glasses*, standard 76 by 25-mm noncorrosive slides. Cover glasses, round or square, clean and free of oil.

7. Reagents

7.1 *Purity of Reagent*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society,

[1] This test method is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

where such specifications are available.[3] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Type I reagent water conforming to Specification D 1193.

7.3 *Formalin*—To prepare the formalin preservative, mix 900 mL of 37 to 40 % aqueous formaldehyde (100 % formalin) with 100 to 150 mL of 20 % surgical detergent solution and 20 to 30 mL of saturated cupric sulfate solution.

7.4 *Lugol's Solution*—An alternative preservative is Lugol's solution. Prepare a stock Lugol's solution by dissolving 600 g of potassium iodide and 40 g of iodine crystals in 1000 mL of water.

8. Precautions

8.1 Formaldehyde vapors are toxic, and the concentrated solution can damage exposed skin or eyes. Wear waterproof gloves and appropriate eye protection when handling concentrated formaldehyde solutions. Work in adequately ventilated areas.

9. Sampling

9.1 Collect the sample in accordance with Classification D 4149.

9.2 Preserve the sample with either formalin or Lugol's solution. If formalin preservative is desired, mix 40 to 50 mL of formalin preservative with each 1000 mL of sample. If Lugol's solution is preferred, mix 37 mL of Lugol's solution with each 1000 mL of sample, and store in the dark.

10. Microscope Calibration

10.1 Mount the ocular micrometer (Whipple grid) in one eyepiece in accordance with the manufacturer's instructions for placement.

10.2 Set up the microscope and place the stage micrometer on the stage with the etched markings upper most.

10.3 Focus on the ruled graduations under low power (100×). Measure and record the dimensions of the Whipple grid to the nearest 0.01 mm. Repeat the procedure for all other objective/ocular combinations suitable for use with the Sedgwick-Rafter cell. Often this is 200× or 250×. At magnifications greater than 100×, the Whipple grid should be measured to the nearest 0.001 mm (American Public Health Association, 1976). Calculate and record the area enclosed by the Whipple grid in square millimetres at each magnification.

11. Pretreatment

11.1 Some samples may require concentration or dilution prior to analysis. The decision to concentrate or dilute is subjective and should be reached only after microscopic examination of the sample. This can be done by preparing a wet mount as follows: Mix the sample gently, then pipet a drop onto a clean microscope slide, and add a cover slip. Examine at 100× for general concentration. If desired, concentration or dilution may be performed by one of the following procedures:

11.2 Sample concentration can be accomplished in many ways; settling is the preferred way. One method is to transfer the entire sample to a graduated cylinder of sufficient capacity, noting the initial volume, and then carefully removing most of the supernatent by siphoning after the algae have settled completely. Allow a time interval for settling of 4 h/cm of depth (Greeson, 1977). Note and record the volume of concentrate. Another method is to weigh the water sample and contents to the nearest 1 g, then allow the algae to settle to the bottom for 4 h/cm of depth. Note and record the initial gross of sample weight. Then remove most of the supernatant by siphoning. The remaining sample and container are weighed again to determine the weight of sample discarded. Assuming an equivalence of weight and volume (1 mL = 1 g), calculate and record the volume of concentrate that will be used in later analysis. Transfer the concentrated sample to another container, then weigh the container to determine the actual concentration factor.

11.3 Dilution of the sample may be necessary to reduce the concentration of suspended sediment that would otherwise obscure the algae during analysis. Occasionally a sample may have a particularly high density of algae and require dilution. No specific guidelines are available to suggest when dilutions should or must be made. The analyst must decide whether or not to dilute based on past experience. If it is decided to dilute, first mix the sample thoroughly but gently by inverting the sample container several times. Pipet a known volume into an appropriate graduated cylinder (usually a 50 or 100-mL cylinder is satisfactory). It is inadvisable to transfer sample volumes less than 5 mL because of the difficulty in accurately measuring very small aliquots of sample. Dilute the sample to the desired point with reagent grade water but, in any case, not beyond the capacity of the graduated cylinder. Record the initial volume (the aliquot that was diluted) and the diluted sample volume.

12. Procedure

12.1 Lay the Sedgwick-Rafter cell on a flat surface with the cover glass placed diagonally across it.

12.2 Mix the sample thoroughly by turning the sample bottle end over end no less than ten times. Avoid shaking the sample as this may cause foaming or damage to delicate algae.

12.3 Remove a 1-mL aliquot using a pipet and transfer it to the Sedgwick-Rafter cell, being careful to avoid getting bubbles under the cover glass. The cover glass must not float above the rim of the cell. Allow the counting cell to stand on a level surface a minimum of 24 min for the algae to settle. Often it is helpful to prepare a wet mount at the same time that can be used for taxonomic identification of the algae at 400× or 950×. For example, an aliquot (50 to 100 mL) of sample can be concentrated by centrifugation or settling, before a useful wet mount can be made.

12.4 Following settling, count the algae either by strip count or random field technique. The strip-count technique involves counting cells within the width of a ocular grid for the entire length of the Sedgwick-Rafter cell. Several such "strips"

[3] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."

comprise the count. This technique is useful for relatively sparse samples. Another technique is to count the algal cells lying within the confines of the ocular grid in randomly spaced fields. This technique is particularly useful for dense samples but is cumbersome for sparse samples. There is no agreement as to the number of algae that must be counted in order to assume a statistically valid sample. Most workers suggest counting a minimum of 100 organisms at the very least.

12.5 The counting procedures are basically the same. Two adjacent sides of the ocular grid are designated "count" sides and the other two "no-count" sides. Count all cells that lie within the grid as well as those which touch a "count" side. Do not count that part of a trichome that extends outside the grid area. When making a random field count, count a minimum of 100 cells or no less than 10 fields, whichever is obtained first, but not less than 10 fields. For strip counts, count a minimum of 100 cells but make at least one complete sweep from side to side. Avoid partial sweeps. Tally each taxonomic type separately.

12.6 Some algae may not settle but instead rise to the underside of the cover glass. These cells should be included in the tally where they occur within the borders of the grid.

12.7 When colonial forms are encountered, it may be impossible to count all the cells because of partial obscuring of underlying cells. In such cases, it is acceptable to calculate the total number of cells lying within the grid by estimation. Similarly, determine the total number of cells in filamentous forms by multiplying the mean cell length by the size of trichome. Count the frustules containing protoplast as having been living at the time of collection. Do not include empty frustules in the tally.

13. Calculations

13.1 Calculate random field count as follows:

$$\text{Cells/mL} = (C)(N/E)(P)(D) \tag{1}$$

$$C = A/G$$

where:
C = Calibration factor,
A = area of S-R cell, mm^2, and
G = field (grid) area, mm^2.

$$P = S/V \tag{2}$$

where:
P = preservative factor,
S = sample plus preservative volume, mL, and
V = sample volume, mL.

$$D = M/O \tag{3}$$

where:
D = concentration or dilutions factor,
M = concentrated or diluted volume, mL, and
O = original sample volume, mL.

13.2 Calculate strip counts as follows:

$$\text{Cells/mL} = (C)(N/E)(P)(D) \tag{4}$$

$$C = A/B$$

where:
B = area of strip, mm^2.

$$P = S/V \tag{5}$$

$$D = M/T$$

where:
T = sample volume prior to concentration per dilution.

14. Report

14.1 Results shall be reported as the number of cells per millilitre for each taxonomic type.

15. Precision and Bias

15.1 Because of the taxonomic complexity of the samples, the true value, and therefore the accuracy, cannot be determined.

15.1.1 The precision of the count is related to the number of organisms counted, and is determined by calculating the square root of the tally. For example: if 100 cells (or units) are encountered in ten fields, the precision of the count would be $100 \pm \sqrt{100} = 100 \pm 10$, or 100 ± 10 %. The final value, in terms of cells (or units) per millilitre can be determined by the appropriate conversion factor. In the example, the precision of the final value would be ± 10 %.