Chlorophyll—a raw water quality parameter

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Utilities that use surface water supplies should measure planktonic algal chlorophyll as a raw water quality parameter on a routine basis. Chlorophyll measurement can be a viable substitute for the more costly and tedious algal counting method used by many utilities. A simplified procedure for chlorophyll measurements is presented, and suggestions are made on how water utilities can correlate the results of these analyses with raw water quality parameters of importance to them. Chlorophyll measurements of the lake or impoundment at or near the water supply intake can provide guidance as to the depth at which the water should be taken from the body of water in order to optimize raw water quality and reduce the cost of treatment.

Eutrophication of domestic water supplies is one of the major causes of raw water quality deterioration. Planktonic algae and other aquatic plants can cause undesirable tastes and odors, reduced filter runs, increased chlorine demand, and sometimes increased trihalomethane (THM) precursor content, all of which can affect the cost of treating the water for domestic use. Traditionally, water utilities have used algal numbers or types (following procedures described in *Standard Methods*[1]) as the basis for adjusting treatment processes to minimize the effects of excessive growths of aquatic plants in the raw water supply. Algal counting is time-consuming, laborious, expensive, and not always accurate. Measuring the concentrations of an algal pigment—chlorophyll—is an alternative approach, which should be considered by water utilities to supplement and eventually to replace detailed algae counting.

Determining the chlorophyll concentration involves extracting the chlorophyll from the algae with acetone and reading its absorbance at specific wavelengths by means of a spectrophotometer. Although there are several deficiencies in the ability of this simple procedure to measure the algal numbers, biomass, and the impact of the algae on raw water

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quality, there are many similar deficiencies associated with using algal counts. Often the correlation is poor between algal counts, presented as either total number of organisms or specific types, and raw water quality problems. The authors believe the correlations that can be established between planktonic algal chlorophyll and the water quality problems of a water supply will be at least as good as, and possibly better than, those currently being used and also will save time and money in laboratory operations. However, microscopic examination of water samples should not be abandoned completely, because certain algae cause more problems than others. Identification of the dominant algal types combined with the simple determination of planktonic algal chlorophyll will provide a better assessment of potential water quality problems than algal quantification-identification alone. Such information also will guide utilities in altering their water treatment programs to produce the highest possible finished water quality at the least possible cost.

Planktonic algal chlorophyll is also an important parameter in the OECD's (Organization for Economic Cooperation and Development) eutrophication modeling approach. This model correlates the nutrient (principally P) load of a body of water with its eutrophication-related water quality characteristics. The use of the model has been described elsewhere.[2-5]

Chlorophyll in water quality assessment

In the past, many persons questioned the importance and the technical validity of chlorophyll measurements in water quality assessment. Admittedly, there are problems with the analytical techniques used. The amount of chlorophyll extracted from algae depends on a variety of factors, including the age and nutritional status of the cell, the solvent(s) used, the specific algae in the sample, and extraction efficiencies. Furthermore, for any given procedure, the same calibration curve (i.e., concentration-response curve) is used for all spectrophotometric measurements. Although calibration curves can vary among instruments, and even for a single instrument from day to day, the chlorophyll determination procedures[1] include the use of standard absorptivities-extinction coefficients that were determined by one individual many years ago and assume that these coefficients can be used with all equipment. Another problem in measuring chlorophyll is that large amounts of suspended solids in some samples tend to sorb a portion of the extracted chlorophyll, producing erroneously low readings. However, even with these problems in measuring chlorophyll, its concentration has considerable usefulness as a parameter for characterizing the water quality of a water supply.

Several years ago, OECD conducted a 5-year study of 200 water bodies to define relationships between nutrient load (primarily P) and eutrophication-related recreational water quality. Data on approximately 30 water bodies (lakes and impoundments) in the United States were evaluated by Rast and Lee.[6] They considered a preliminary correlation[7] between the annual P loads normalized by the water bodies' mean depths and hydraulic residence times and the average planktonic algal chlorophyll concentration for a group of water bodies. Figure 1A shows the correlation between P load and chlorophyll,[6,8] as expanded by Jones and Lee[2] with the inclusion of data for about 40 more water bodies in the United States. Although there is scatter about the line of best fit, this correlation has demonstrated predictive capability[9] and sufficient resolution for decision making. The scatter, based on the work of Rast et al,[9] does not appear to be related significantly to deficiencies or difficulties with analytical methods. Based on data from the literature for several hundred water bodies, Rast and Lee[6] developed a relationship between chlorophyll and Secchi depth (water clarity) that led to the development of the correlation between normalized P load and Secchi depth (Figure 1B). This model is restricted to those water bodies having only moderate amounts of inorganic turbidity, because it can relate only P load to the impact of planktonic algae on water clarity. Undoubtedly some of the scatter in Figure 1B is due to differences in the amounts of inorganic turbidity in the water bodies. If this model was used to estimate Secchi depth in a water body having large amounts of erosional material or other suspended, nonalgal material, the predicted Secchi depth would be expected to be greater than that actually found. Figure 1C, the correlation between normalized P load and hypolimnetic oxygen depletion rate, also is based on the existence of a relationship between the amount of planktonic algae (chlorophyll concentration) and the decrease in hypolimnetic oxygen concentration owing to the decomposition of these algae.

The relationships shown in Figure 1A are based on average summer response. Algal blooms often exhibit a high peak in chlorophyll concentration lasting from a few days to a week or so, followed by death and rapid settling of the algae to the bottom of the water body. During periods of high chlorophyll levels, water utilities can experience highly deteriorated raw water quality. In some cases, these problems make it difficult for the water plant operators to add sufficient chemicals, such as activated carbon, to maintain a high-quality finished water. Jones et al[10] found that the worst-case conditions—maximum chlorophyll—can be estimated to be about 1.7 times the mean chlorophyll level in the summer. This relationship can be of value to water utilities in sizing the treatment works and apparatus to cover expected worst-case conditions. Lee et al[11] discussed how each of these parameters should be used in evaluating the overall trophic state of a water body.

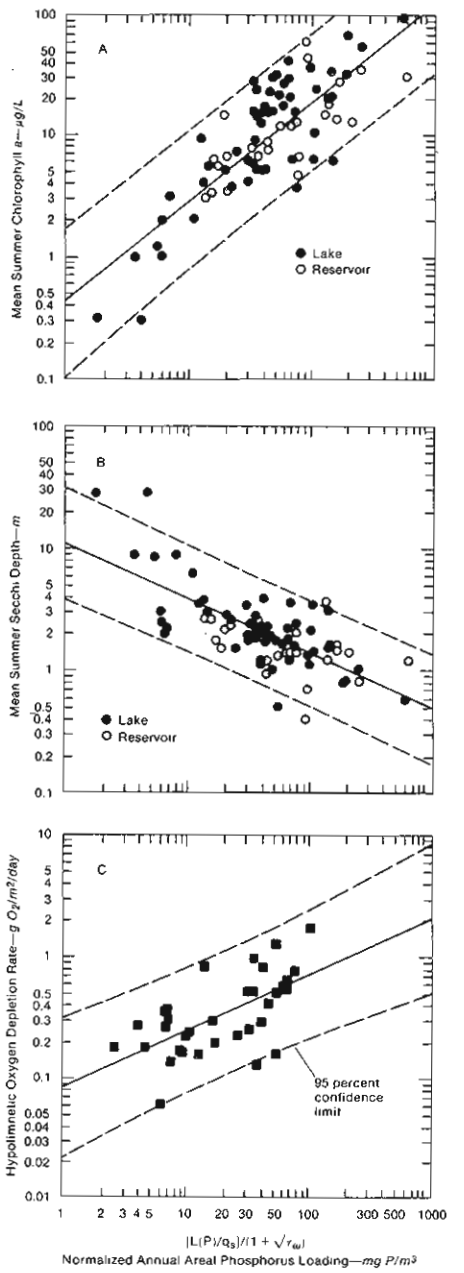

Figure 1. Updated phosphorus load-eutrophication-related water quality response relationships for bodies of water in the United States

After Jones and Lee;[2] L(P) = areal annual phosphorus load (mg P/m²/year); q_s = mean depth ÷ hydraulic residence time = $\bar{z}/\tau_\omega$ (m/year); τ_ω = hydraulic residence time (years)

TABLE 1
Guide to acceptable precision

Chlorophyll Concentration Range μg/L	Maximum Acceptable Difference Between Duplicate Analyses μg/L
<5	1
5-10	2
10-20	3
20-50	5
>50	10

Chlorophyll as a water quality parameter. Planktonic algal chlorophyll is becoming recognized as a parameter of choice for describing water quality problems associated with planktonic algae in domestic water supplies. Archibald and Lee[12] found good correlation between the raw water quality in a water supply reservoir for the city of Dallas, Texas, and the planktonic algal chlorophyll in the surface waters of this reservoir. They also found that the reservoir followed the P load-response relationships[2,6] for chlorophyll. The normalized P load to the main body of the reservoir reliably predicted the planktonic algal chlorophyll in this part of the reservoir. By using this approach, they were able to predict the impact of the increased population in the watershed on the eutrophication-related water quality of this reservoir.

Another example of using chlorophyll as a parameter of water quality was provided by the work of Horstman et al[13] on Lake Dillon, a water supply reservoir for the city of Denver, Colo. By using the P load-response relationships,[2,6] they found that reducing P to 1 mg P/L in effluents from nearby communities' domestic wastewater treatment plants was necessary to protect water quality in this reservoir.

For the OECD model, and modifications of it, to be of greatest value to water utilities, more detailed information on the relationship between chlorophyll concentrations and raw water quality problems needs to be collected. Good correlations are available for relating chlorophyll to P load (a parameter that generally can be controlled) and to changes in P load. Moreover, the generalized couplings between chlorophyll and recreational water quality are known. With the additional coupling of chlorophyll to raw water quality, general changes in domestic water treatment costs and procedures can be estimated from alterations in P load. As indicated previously, periods of maximum raw water quality problems will be signaled by rising chlorophyll levels, and the extent of the problem—requirements for added treatment—can be predicted. Until more detailed correlations can be made, however, it will be important for utilities to measure chlorophyll, determine dominant algae types, and record periods of water quality problems.

Analytical procedure

Some water utility personnel have indicated to the authors that they find the chlorophyll procedure described in *Standard Methods*[1] difficult to follow and use. The authors believe the difficulties are the result of numerous options provided in the procedure. Although all of the options have appropriate applications, the simple, straightforward analytical procedure for chlorophyll is best in routine monitoring of domestic water supplies.

Sample collection. Samples of water should be collected near the intake at midday every two weeks during late fall and winter. In early spring, when the algal numbers (chlorophyll) in the raw water supply first increase significantly, samples should be collected every week; collection should be continued at this level throughout the late spring, summer, and early fall. For some water supplies, such as rivers or large bodies of water in which fairly rapid changes in algal numbers can take place within a few days, samples should be collected every two to three days. Samples must be kept in the dark and refrigerated until analysis; samples should be analyzed within about 24 hours of collection. Although *Standard Methods*[1] indicates that chlorophyll samples may be stored frozen, the authors have encountered problems with this technique. Each of the major algal types in a water supply should be present in both frozen and fresh samples. Therefore, samples should not be frozen unless investigation has shown that results from analyses of frozen samples compare favorably with results from analyses of fresh samples. Moreover, one should not assume that even though freezing has been found to be a satisfactory preservation method for samples collected in the spring, it will also work for samples collected in the summer.

Simplified analytical procedure for chlorophyll. The analytical procedure for chlorophyll is similar to that presented in *Standard Methods*.[1]

1. Filter 50 to 500 mL of a thoroughly mixed water sample through a 0.45-μm-pore-diameter membrane filter. Record the volume of sample filtered.

The sample volume needed depends on the amount of chlorophyll in the sample. The sample size should be sufficient to produce an absorbance (optical density) reading between about 0.1 and 0.7, but ideally at about 0.3, at 663 nm. Until this judgment about the sample size can be made visually, the analyst may run replicate analyses on several sample volumes. The samples should be prepared for replicate (at the least) analyses.

A distilled-water blank should be processed along with each set of samples. The volume of water filtered should be about the same as the volume filtered for each of the samples.

2. When a few millilitres of unfiltered sample are left in the filter funnel, add 0.2 mL of a saturated magnesium carbonate solution. Before pipetting the solution, shake it to suspend the precipitated magnesium carbonate.

The solution is prepared by adding 1 g of finely powdered magnesium carbonate to 100 mL of distilled water. This solution also should be added to the distilled-water blank during filtration.

3. After filtration is complete, carefully remove the filter from the holder and place it in a 15-mL, graduated, screw-cap centrifuge tube.

Some individuals prefer to macerate the sample and filter in a tissue grinder. For some algae, maceration will yield more extractable chlorophyll, because the chlorophyll may be more readily and completely released from the cells. However, a variety of techniques, many of which give slightly differing results, were used in collecting the chlorophyll data on which the P load-response models[2,6] are based. The difference in results caused by maceration of the sample appears unimportant. Although more reproducible results will be obtained if a tissue grinder is used, the differences in results from the two techniques are not sufficient to warrant the purchase of a tissue grinder.

4. Add 5 mL of 90 percent acetone (v/v) to the centrifuge tube. Tighten the screw cap, wrap the tube in aluminum foil to protect its contents from light, and shake the tube vigorously. Place the centrifuge tubes in the dark, at 4°C, for approximately 24 hours.

The acetone is prepared by mixing 90 parts of reagent-grade acetone with 10 parts of distilled water (v/v).

The procedure is modified frequently by storing the extractions overnight at room temperature. Although storing the extracts at 4°C is desirable, it is not mandatory. If refrigeration space is not available, the extracts can be incubated at room temperature. However, the extracts must be stored in the dark.

The procedures adopted for tissue grinding and storage must be followed consistently, and notations should be made in the laboratory notebook as to techniques followed. If a change in procedure is made at some time in the future, then the samples should be processed by using both techniques for a period of time (i.e., over one year) to see if significant differences in results are found.

5. At the end of the incubation period (about 24 hours), remove the aluminum foil from the centrifuge tubes. Add 90 percent acetone to fill the tube to about the 15-mL mark (see step 6). Centrifuge in a table-model, clinical centrifuge at 500 g for 20 min.

The volume (15 mL) in the centrifuge tube is usually what is required to fill a 5-cm-path-length cell.

6. Read and record the volume of acetone extract in the centrifuge tube. Carefully decant the supernatant from one tube into a spectrophotometric cell with a 5- or 10-cm path length. Determine the absorbance of the supernatant at wavelengths of 750, 663, 645, and 630 nm, after using the 90 percent acetone to set the zero for each wavelength. Repeat these steps for each supernatant and blank.

The light path for such spectrophotometric readings should be selected so that the absorbance at 663 nm is between about 0.1 and 0.7, but ideally around 0.3. The longer light paths are needed for water samples with low planktonic algal chlorophyll. If the 5- and 10-cm cells are not available, then an additional volume of sample may be filtered to compensate for the cells with shorter light paths.

7. To correct for turbidity, subtract the absorbance readings at 750 nm from the readings at the other three wavelengths. Using the corrected absorbance values for each sample, calculate C_a as follows:

$$C_a = 11.64A_{663} - 2.16A_{645} + 0.10A_{630}$$

where C_a is the concentration of chlorophyll a in the extract and A is the corrected absorbance at a particular wavelength (given as the subscript). Use the value of C_a to determine the concentration of chlorophyll a:

$$\text{(chlorophyll } a\text{, } \mu\text{g/L)} = \frac{C_a \times \text{(volume of extract, mL)}}{\text{(volume of sample, L) (light path, cm)}}$$

The results should be reported as the average of the replicate samples. If the duplicate values for a single sample differ by more than shown in Table 1, additional samples should be run in subsequent analyses to reduce scatter in the data.

Any background turbidity is subtracted out when the absorbance reading at 750 nm is subtracted from the other absorbance readings. Therefore, the blank that is carried through the procedure is not used in calculating the concentration of chlorophyll; it serves only to assure the technician that no gross contamination is occurring. If measurable readings are obtained in the blank, the source of contamination or operator error should be identified and eliminated.

Discussion

As indicated earlier, the authors recommend that planktonic algal chlorophyll measurements be correlated with various eutrophication-related parameters, including algae, tastes and odors (i.e., raw water threshold odor intensity), THM content, length of filter runs, activated carbon usage, and chlorine demand. For example, a graph of threshold odor number (y-axis) as a function of chlorophyll concentration (x-axis) eventually will show a line of best fit (i.e., correlation). For some response parameters, such as length of filter run, it may be more appropriate to determine average values for a given period. The details of developing correlations will depend to some extent on a water plant's monitoring program. It is likely that good correlations between algal chlorophyll and certain parameters will be found only during limited periods of the year, e.g., tastes and odors (threshold odor number) during the summer.

Furthermore, each water supply will have characteristic relationships; correlations established for one water supply system will not necessarily be applicable to another because of differences in algal types, depth at which the water intake is located, and overall productivity of the raw water, among others. However, these differences should not discourage the operator or manager from using planktonic algal chlorophyll as a water quality parameter. About three years of data should be accumulated before determining that this parameter is not a useful tool for a particular water supply.

Also, one should remember how much treatment control depends on the accuracy or precision of chlorophyll data as well as algal-count data. Although correlations between treatment needs and chlorophyll level may be rough, they are expected to be at least as good as those based on algal counts and can be obtained with less effort and expense. It is likely that after several years of experience, water utilities can substitute chlorophyll measurements for the counting procedures they frequently use now.

For those water utilities that are using rivers as the raw water source, planktonic algal chlorophyll may correlate well with certain taste and odor problems. It is the experience of one of the authors that some of the taste and odor problems of the upper Ohio River, which were frequently blamed on industrial dumping, correlated well with planktonic algal chlorophyll. Algae that grew in various headwater reservoirs were released downstream in "slugs" as reservoir waters were released.

In addition to correlating chlorophyll with eutrophication-related response parameters, consideration should be given to developing correlations between chlorophyll concentration and overall cost of plant operation, focusing on those periods of elevated chlorophyll levels. With this type of information it may be possible to develop chlorophyll-cost relationships that will enable a water utility to begin assessing the additional costs associated with eutrophication-related deterioration in raw water quality.

In addition to using chlorophyll measurements to characterize the quality of a treatment plant's intake water, chlorophyll measurements can be helpful in characterizing the reservoir or lake and in determining, for example, the location of the water intake. Details of a sampling program that water utilities can use in order to characterize the raw water supply[3,4,5] include taking a set of samples near the deepest point in the lake or reservoir. Lee and Harlin[14] reviewed the influence of intake depth in lakes and reservoirs on water quality. As they stated, if the body of water stratifies thermally and the hypolimnion contains oxygen that even in many eutrophic lakes occurs throughout a substantial part of the summer, then the best quality water is typically that taken from the hypolimnion below the thermocline. As soon as the oxygen starts to decrease significantly in the hypolimnion, however, manganese is released, and, as the oxygen level decreases to zero, iron and sulfide may be released, making hypolimnetic waters difficult to use for domestic water supplies. In most water bodies during the summer, the algae, which can be measured by chlorophyll, usually occur in the greatest numbers in the epilimnion and in the greatest concentrations a few metres below the surface. If the hypolimnion is anoxic, it may be possible to select epilimnetic waters that have the least amount of algae and potentially fewer water quality problems by measuring the distribution of chlorophyll in the water column. For those water bodies in which the intake works has a bridge to the shore, vertical distribution of chlorophyll can be investigated by collecting samples from various depths along the bridge at the intake works. If the intake works is located offshore, then sampling will have to be done from a boat.

Attempts should be made to compare the chlorophyll profile at the intake works with the level in the routine samples received in the laboratory. Eventually when a general relationship is established, depth of intake (where variable intake is possible) can be adjusted, based on the composition of the raw water and chlorophyll and temperature relationships. For some utilities, the extra work involved in control of the raw water quality can pay for itself several times over in terms of reduced chemical costs for treatment.

Conclusions

The authors recommend the use of chlorophyll measurements by water utilities as part of determining the quality of raw water supplies. Because measuring chlorophyll concentrations is simpler and less expensive than algal counting, eventually it may replace algal counting. There appear to be several situations in which correlations between chlorophyll concentration and other water quality parameters may be used to make decisions regarding water treatment. Direct applications of these correlations to treatment

can include adjusting intake depth and eliminating taste and odor problems.

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References

1. *Standard Methods for the Examination of Water and Wastewater.* APHA, AWWA, and WPCF. Washington, D.C. (15th ed., 1981).
2. JONES, R.A. & LEE, G.F. Recent Advances in Assessing the Impact of Phosphorus in Eutrophication-Related Water Quality. *Jour. Water Res.*, 16:503 (1982).
3. LEE, G.F. & JONES, R.A. Study Program for Development of Information for Use of OECD Modeling in Water Quality Management. AWWA Quality Control in Reservoirs Committee Report (1981). Approval pending.
4. LEE, G.F.; JONES, R.A.; & RAST, W. Evaluation of Nitrogen and Phosphorus Loads to Water Supply Reservoirs. AWWA Quality Control in Reservoirs Committee Report (1981). Approval pending.
5. LEE, G.F. & JONES, R.A. Determination of Nutrient Limiting Maximum Algal Biomass in Waterbodies. AWWA Quality Control in Reservoirs Committee Report (1981). Approval pending.
6. RAST, W. & LEE, G.F. Summary Analysis of the North American (US Portion) OECD Eutrophication Project: Nutrient Loading-Lake Response Relationships and Trophic State Indices. EPA Rept. EPA-600/3-78-008. USEPA, Corvallis, Ore. (1978).
7. VOLLENWEIDER, R.A. Input-Output Models, with Special Reference to the Phosphorus Loading Concept in Limnology. *Schweiz A. Hydrol.*, 37:53 (1975).
8. LEE, G.F.; RAST, W.; & JONES, R.A. Eutrophication of Waterbodies: Insights for an Age-Old Problem. *Envir. Science & Technol.*, 12:900 (1978).
9. RAST, W.; LEE, G.F.; & JONES, R.A. Predictive Capability of US OECD Phosphorus Loading-Eutrophication Response Models. *Jour. Water Pollution Control Federation* (in press).
10. JONES, R.A.; RAST, W.; & LEE, G.F. Relationship Between Mean and Maximum Chlorophyll *a* Concentrations in Lakes. *Envir. Science & Technol.*, 13:869 (1979).
11. LEE, G.F.; JONES, R.A. ; & RAST, W. Alternative Approach to Trophic State Classification for Water Quality Management. Unpublished.
12. ARCHIBALD, E.M. & LEE, G.F. Application fo the OECD Eutrophication Modeling Approach to Lake Ray Hubbard, Texas. *Jour. AWWA*, 73:590 (1981).
13. HORSTMAN, H.K.; LEE, G.F.; & JONES, R.A. Eutrophication-Related Water Quality in Dillon Reservoir. Proc. Envir. Engrg. Conf., New York (1980).
14. LEE, G.F. & HARLIN, C.C. Effect of Intake Location on Water Quality. *Industrial Water Engrg.*, 2:36 (1965).

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