Determination of Nutrient Limiting Maximum Algal Biomass in Waterbodies

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INTRODUCTION

The concept of focusing eutrophication control programs on the nutrient or factor governing the maximum algal biomass stems from Liebig's Law of the Minimum which states that the growth of an organism is limited by the substance or other factor which is available to it in the least quantity relative to its needs for growth or reproduction. Specifically, in a simplistic sense, algae will grow until one of the items required for growth is not available in sufficient quantities. While there is a myriad of factors which could limit the maximum algal biomass developed in a waterbody, in most waterbodies it is limited by nitrogen or phosphorus.

This paper was developed as a guide for water quality managers to determine the nutrient which limits algal biomass in waterbodies, in connection with eutrophication management. It also addresses the appropriate use of this information in the application of the Vollenweider-OECD eutrophication modeling approach.

ALGAL BIOASSAYS

One of the procedures frequently used to evaluate the limiting nutrient in a water of concern is the algal bioassay. Procedures such as those outlined by the US EPA (1971, 1974), APHA et al. (1985), Sridharan and Lee (1977), and Miller et al. (1978) are typically used. This test is sometimes referred to as an algal growth potential test. To conduct algal bioassays, surface (0.5- to 1-m depth) water samples are collected during the period of eutrophication-related water quality concern. If a standard test alga such as *Selenastrum* is used, the water samples are generally filtered through 0.45 u pore size membrane filters. There is discussion in the literature about the advisability of using a standard alga in a nutrient limitation study rather than native populations because of differences in what may limit growth of one type of alga versus another. While this may be of ecological significance in species selection, it is usually of limited concern in eutrophication management since with few exceptions the behavior of the test alga *Selenastrum* seems to be adequately representative of that of the majority of algae typically comprising the mixed-algae natural system, for water quality management.

To aliquots of the filtered water sample, known quantities (spikes) of nitrogen, phosphorus, and trace elements are added in various strengths, singly and in combination, along with a known quantity of the test algae. A series of standard nutrient solutions is also prepared to contain various known concentrations of available nitrogen (ammonia and nitrate) and phosphorus (soluble orthophosphate) and the designated quantity of algae. The cultures are incubated under appropriate (usually optimum) light and temperature conditions; the amounts of algae in the samples and standard solutions are measured over a period of a week or two, until growth plateaus are reached.

The effects of the nutrients on the maximum algal biomass present at the plateaus indicate which nutrient is controlling maximum biomass in the water sample under the conditions of the test. If, for example, the addition of phosphorus to the water sample stimulates a higher maximum biomass production than that found in the unspiked sample, and the addition of available nitrogen does not, phosphorus is assumed to be the limiting nutrient for that condition.

Caution must be exercised when using this approach since laboratory light and temperature conditions are typically set at optima for the test organism, which may be markedly different from those found in the waterbody. Therefore, the results of an algal bioassay as described above may show phosphorus limitation, whereas in the waterbody, the phosphorus may never be decreased to limiting levels because the amount of light restricts the ultimate biomass produced, or the rate of supply of phosphorus to the area of interest is sufficiently great relative to the algal growth rate to preclude the reduction of P to limiting levels.

The amounts of the nutrient spikes added to the water samples should be kept relatively small. If large spikes are used, it is possible to drive the laboratory system to N or P limitation rather than to assess which is limiting in the waterbody.

It is important that algal assays for limiting nutrients be run on water samples collected during the period of water quality concern, i.e., during periods of potential or realized impairment of beneficial uses of the water. What may be limiting during less critical times may not be limiting during times of water quality concern. Similarly, the samples should be collected from the area of the waterbody experiencing the eutrophication-related water quality problem. For example, in some waterbodies, the pertinent characteristics in an arm may be substantially different from those in the main body; if it is the water quality in the arm that is of concern, then the samples should be collected from that area. The presence of arms or extensions of the main waterbody is particularly common in reservoirs and should be recognized, as discussed, when evaluating the limiting nutrient or water quality characteristics of such waterbodies.

Differences in algal growth limiting factors are also found between the nearshore and open waters of large waterbodies, such as the Great Lakes. There are situations, however, where the water quality problems materialize in areas away from the source of the problem. Often, problems of algal accumulation on beaches and nearshore areas are caused by algae that had grown in the open waters but were blown to shore. In those cases, the open water should be the focal point for assessment of limiting nutrients.

The type of algal assay described above not only will provide an indication of which nutrient is limiting algal biomass production in a waterbody, but will also indicate if there is something in the water which is toxic to algae. If a toxicant is present, the growth in the test systems will be less than that in the standard systems containing the same amounts of nutrients. Further, as discussed by Lee et al. (1980), a modification of this algal assay procedure can be used to evaluate the availability of nutrient forms found in a water.

There are also techniques available for determining whether algae in a waterbody have grown under conditions of limiting or surplus N or P. These include analysis of the cells for N and P content, analysis for enzymes such as phosphatases that are produced under conditions of low availability of soluble orthophosphates, determination of ammonia uptake rates, and analysis for acetylene reduction ability of nitrogen-fixing algae. Further information on these techniques is available in Stewart et al. (1970) and Fitzgerald (1972). While these approaches have some utility for assessing nutrient limitation under certain conditions, the results of such tests are often confusing in that in mixed populations of algae, a non-nitrogen-fixing algal growth may be limited by nitrogen while in the same water another alga having the ability to fix atmospheric nitrogen, growth could be limited by phosphorus. It should be noted, as found by Torrey and Lee (1976), that algae types that can fix atmospheric N do not always do so, even under conditions of low concentrations of available nitrogen in the water.

The large amount of work that has been done during the past 10 years in determining limited nutrients in waterbodies around the world has shown that essentially the same limiting nutrient information that can be derived from algal assays can be obtained much more readily and less expensively using chemical measurement techniques. These techniques are described below.

CHEMICAL ANALYSIS

A simpler method to determine what is limiting algal growth is one based on knowledge of the stoichiometric uptake of nutrients by algae. Surface water samples (0.5- to 1-m depth) are collected during critical periods of maximum algal biomass and analyzed chemically for available nitrogen (ammonia and nitrate) and available phosphorus (soluble orthophosphate). This will provide an assessment of the amounts of nutrients that are still available in excess amounts after the peak biomass has occurred; those present in excess amounts relative to the amounts needed for growth are not limiting growth, i.e., the amount of algal available nutrients present in the water is sufficient to allow the algae to grow at a rate independent of the concentration of the nutrient. These concentrations are generally somewhat above the half saturation constant in the Michaelis-Menton-Monod evaluation. Generally, if the soluble ortho P concentration is 0.002 mg P/l or lower, phosphorus would be considered limiting. If the available nitrogen concentration is less than about 0.015 mg N/l, nitrogen would be considered to be limiting. Under some conditions, both nutrients may be present in growth-limiting amounts, and it may be a combination of these nutrients which is limiting maximum biomass development. In some waterbodies, both may be present in amounts greater than these levels, in which case some factor other than nitrogen or phosphorus concentrations, such as light or a toxic substance, would be limiting maximum algal production.

Another approach used in the chemical assessment of limiting nutrients is the N to P ratio, the ratio of the concentration of available nitrogen (ammonia and nitrate) to that of available phosphorus (soluble orthophosphate) in a water sample collected during the period of water quality concern. Algae generally use nitrogen and phosphorus in fairly constant proportions, on the order of 16 to 1 on an atomic basis (7.5 to 1 on a mg/l basis). It is, however, not a narrow range about 16:1; the atomic ratio can range from about 5:1 to 25:1 (Vollenweider, 1985). Thus, if the ratio of concentrations is substantially larger than 7.5 to 1, phosphorus is potentially limiting since the algae, drawing N and P out of the water at a ratio of approximately 7.5:1, would likely run out of phosphorus before nitrogen. If the ratio is substantially smaller than 7.5 to 1, nitrogen is potentially limiting. Whether or not N or P is actually limiting depends on the relative rates of supply of available forms of N and P, and on the actual concentrations of the available forms of N and P as discussed below.

While some individuals attempt to use the N to P ratio alone in comparison with the 16:1 atomic N:P ratio to determine the limiting nutrient, it must be used in conjunction with the available N and P concentrations in the water. The N to P ratio will always show N or P or both N and P limitation because the ratio will either be greater than, equal to, or less than 16:1. The actual concentrations, however, may show that either or neither, in actuality, limit growth.

Some investigators make the mistake of collecting water samples in the spring for N to P ratio determination in an attempt to assess what will be limiting maximum algal biomass production in the summer. This is not appropriate unless the rates of nutrient supply, mineralization, algal growth, and nutrient concentrations remain constant over the spring-to-summer period, which is an unlikely situation. Examples of the use of N to P ratios and absolute concentrations of available nutrients in assessing limiting nutrients are provided by Rast and Lee (1978).

Another common mistake made by investigators, such as Smith (1979), in attempting to determine the limiting nutrient, is the use of the ratio of the concentrations of total N to total P. This approach is technically invalid and frequently leads to errors in assessing the limiting nutrient since, in some waterbodies, especially reservoirs, parts of the total N and total P content of the water are not related to phytoplankton biomass, e.g., may be associated with inorganic particulate matter. In these instances appreciable changes in the total concentration of the limiting nutrient can occur without affecting maximum algal biomass production.

USE OF LIMITING NUTRIENT CONCEPT IN EUTROPHICATION MANAGEMENT

As part of his eutrophication modeling work, Vollenweider (1976) developed a statistical relationship between planktonic algal biomass in a waterbody as measured by chlorophyll, and the P load to the waterbody normalized by its hydrological and morphological characteristics. This normalized P loading was theoretically equivalent to the average in-lake P concentration. Jones and Lee (1986) have substantiated this load--response relationship for more than 500 waterbodies around the world. As discussed by Lee et al. (1978), Vollenweider (1979), Jones and Lee (1982), Rast et al. (1983), Jones and Lee (1986), and Lee and Jones (1991a), the Vollenweider-OECD eutrophication modeling approach can be used to predict eutrophication-related water quality of a waterbody in terms of chlorophyll concentration, Secchi depth (water clarity), hypolimnetic oxygen depletion rate, fish yield, and primary productivity, based on the normalized P load to the waterbody. It has a demonstrated capability for predicting the impact of altered land use and phosphorus management programs on the beneficial uses of the water, e.g., domestic and industrial water supply, swimming, boating, and fishing.

Because of its relating algal biomass to phosphorus, the Vollenweider-OECD P load--response relationships were originally believed to be only applicable to waterbodies in which P was limiting algal biomass. However, based on the review conducted by the authors of the Vollenweider-OECD P load--response relationships, it appears that this is not a necessary condition (Jones and Lee, 1986). The studies of the authors have shown that the Vollenweider-OECD eutrophication modeling approach is also applicable to many lakes and reservoirs with growing season soluble orthophosphate concentrations greater than 100 ug P/l, to as great as 500 ug P/l, well in excess of limiting levels. According to Vollenweider (1985) this appears to be related to the interrelationships in nutrient dynamics in controlling algal growth. Some investigators (e.g., Schindler, 1977) have found that even though nitrogen limitation is indicated by chemical or biological evaluation, phosphorus in actuality plays the lead role. It has also been found that while it has been generally assumed that marine open waters are nitrogen-limited, nearshore marine and estuarine waters in some areas appear to be P-limited during the summer growing season (Haskins, 1985, Lee and Jones, 1981, Lee and Jones, 1987, and Lee and Jones, 1989). Algal biomass production in these waters has been found to be responsive to P load reductions as expected based on the Vollenweider-OECD P load--response relationships (Jones and Lee, 1986).

Even in those instances where phosphorus clearly appears not to be limiting algal growth, it may be more appropriate to focus on phosphorus control than on nitrogen control. The former is usually more convenient and less expensive, and will result in decreases in planktonic algae once the phosphorus concentration has been decreased sufficiently. This is discussed further by Jones and Lee (1982) and Lee and Jones (1988a).

Lee and Jones (1991b) have found that there are some waterbodies, such as Lake Tahoe in California, where there is surplus phosphorus compared to nitrogen. Under these conditions, reducing the phosphorus input to the waterbody will only affect planktonic algal biomass under conditions where sufficient reduction takes place to make phosphorus a growth rate limiting constituent.

Lee and Jones (1988b) have described the minimum monitoring program necessary to collect the information needed to determine the limiting nutrient and to apply the Vollenweider-OECD eutrophication modeling approach to waterbodies. Their discussion should be consulted for information on selecting sampling locations, appropriate analytical methods, etc.

It is important to emphasize that the determination of the limiting nutrient must focus on the period(s) of peak biomass that is coincident with greatest impairment of beneficial uses of the water. The approach that has been used in some investigations, such as the US EPA National Eutrophication Survey (NES) conducted in the 1970's, of determining N to P ratios at various times of the year for the purpose of predicting the limiting nutrient during the summer, is inappropriate. As noted above, the limiting nutrient can change over an annual cycle. Furthermore, the N to P ratio alone is not a valid indicator of limiting nutrient; the absolute concentrations of a nutrient during the critical time of measurement must also be at growth-limiting values for limitation by that nutrient to actually occur. The US EPA NES program did not focus its assessment of nutrient limitation on the period of peak planktonic algal biomass, nor did it tie N to P ratios to nutrient concentrations. In addition, the data reduction in the NES program involved the averaging of N to P ratios over time and at various sampling locations in the waterbodies. This approach is not valid because the rates of supply and utilization of nutrients change with time and between locations. Therefore, because of these deficiencies in data collection and handling, much of the nutrient limitation information generated during the NES study is unreliable and has led to incorrect conclusions being drawn regarding limiting nutrients in the NES waterbodies.

Some waterbodies will experience a substantial peak in algal biomass production at times other than the summer growing season. For example, Archibald and Lee (1981) found that the largest peak in algal biomass in Lake Ray Hubbard, a water supply impoundment near Dallas, TX, occurred in the winter. However, this peak did not significantly impair the use of this water for domestic water supply purposes. On the other hand, during the period of maximum algal biomass in the summer, the Dallas Water Utility had to temporarily terminate the use of this water supply because of severe taste and odor problems. These two peak biomass periods were controlled by different factors. It is important to understand that the factor which limits peak algal biomass during one period of the year may not limit peak biomass during another period of the year, and that sampling and management programs should be focused accordingly.

One of the most common mistakes made in conducting nutrient load--eutrophication response studies is the use of an analytical method for soluble orthophosphate that has insufficient sensitivity. It is mandatory that soluble orthophosphate levels at least as low as 2 ug P/l be accurately detectable. This level can readily be attained using procedures described by Lee and Jones (1988b).

CONCLUSIONS

While a variety of techniques are available for assessing the algal-limiting nutrient(s) in a waterbody, the procedure that appears to be most reliable and readily usable in association with water quality management is the measurement of algal available forms of N and P at the time of maximum algal biomass during the time(s) of water quality concern. Caution must be exercised in extrapolating the results obtained by this or other techniques, however, to times of the year or parts of the waterbody other than that time and place sampled. The Vollenweider-OECD eutrophication modeling approach has been found to be applicable to waterbodies in which P is not limiting algal biomass production as detected by these methods.

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