Nutrient Limitation on Algal Growth in the Eau Gallie River

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Because algal growth is a major pollution problem and is believed to be caused by large quantities of nutrients injected into the aquatic environment (1), current investigations have centered upon the relationship between algal growth and the distribution of phosphorus and nitrogen in natural water systems. The Eau Gallie River, which is part of the Indian River Complex located in East Central Florida, contains excessive growth of fibrous algae, vascular plants such as water hyacinths (Eichornia pontederiacae), and cattails (Typha alba). This report includes results of a study made to determine a relationship between algal growth and nutrient uptake, and a most probable limiting element in the growth of these algae.

EXPERIMENTAL METHODS

The growth, nutrient uptake, and adaptability of algal species taken primarily from water adjacent to the river bank were observed in the following growth media: (1) 100 percent inorganic nutrient solution prepared according to Hoagland (2); (2) 99 percent inorganic nutrient solution plus 1 percent soil extract (the latter consisted of an autoclaved mixture of garden soil and distilled water (3); (3) 99 percent autoclaved river water plus 1 percent soil extract; (4) 99 percent unsterilized river water plus 1 percent soil extract; (5) 100 percent autoclaved river water; and (6) 100 percent unsterilized river water. Based upon the observations: (1) the predominantly inorganic nutrient solutions were unable to sustain growth of any algal species collected; (2) the "catalytic" effect of soil extract on algal growth was readily apparent; and (3) all predominantly river water solutions proved to be excellent growth media in terms of algal adaptability and growth, the 99 percent river water plus 1 percent soil extract solution was selected as the final test medium.

The algal species involved in the final experimentation

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were identified as Vaucheria spp, Cladophora spp, and Spirogyra spp. Approximately 95 percent pure unialgal strains were attained utilizing a modified dilution technique (2). After sufficient quantities of each algal species had been cultivated, they were consolidated into two groups of similar mass and composition as illustrated in Table 1. One algal mixture was transferred into two liters of sterile culture medium while the other mass was translocated into an equal volume of unsterile growth medium, and monitoring of algal growth/nutrient uptake was commenced.

TABLE 1

COMPOSITION OF ALGAL MASSES

Algae Type	Unsterile Culture		Sterile Culture	
	Mass (gms)	Per Cent Composition	Mass (gms)	Per Cent Composition
Vaucheria spp	4.015	39.8	4.365	46.7
Cladophora spp	3.413	33.8	3.084	33.0
Spirogyra spp	2.672	26.4	1.901	20.3

Each algal culture was grown in a sterilized gallon plastic container chosen to prevent silica contamination that could result from a glass container (2). The algal growth variables such as pH, type of algae, light intensity, temperature, nutrient concentrations, and photoperiod were controlled to closely simulate actual river conditions. The initial nutrient values were adjusted to be within one standard deviation of the overall average river values previously determined during a three-month field study (4).

A 20-watt, soft, white fluorescent light in conjunction with two, 15-watt bulbs served as the light source. The 15-watt bulbs were tinted blue to more nearly simulate the spectral distribution of sunlight. The light/culture media distance was adjusted to assure the light intensity incident at the surface to be 185 foot-candles (5, 6). The surface light intensity and volume were maintained constant throughout the experiment by the periodic addition of distilled H_2O. External light interference was eliminated. Light intensity was monitored by a Weston illumination meter.

Temperature of the media was measured with a standard laboratory thermometer and was maintained at 25°C by use of an ice water bath. The establishment of a light/dark interval of 16/8 hours also assisted in temperature control. The culture media were magnetically stirred during the photoperiod. Phosphate, nitrate, and pH were all determined according to Standard Methods. The apparent nitrogen and phosphorus uptake by each algal culture is illustrated in Figures 1 and 2, respectively. The pH variation with algal growth is shown in Figure 3.

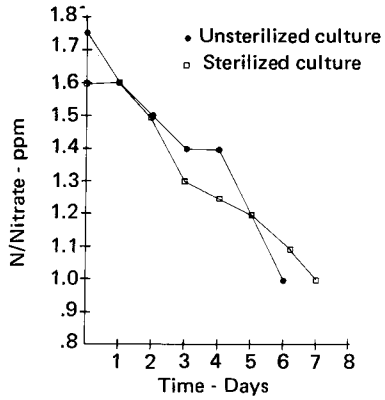

FIGURE 1. Apparent nitrogen uptake.

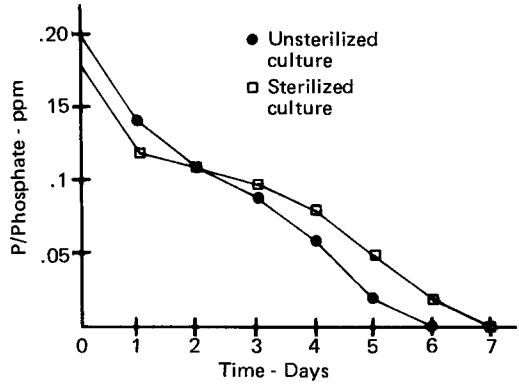

FIGURE 2. Apparent phosphate uptake by algae cultures.

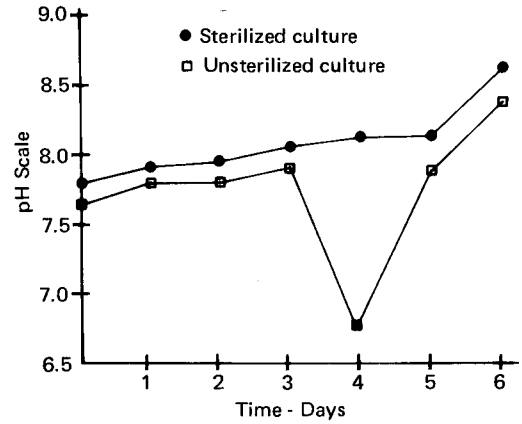

FIGURE 3. Variation of pH with algal growth.

The larger quantity of biomass produced in the unsterile medium (Table 2) is indicative of an increase in biological activity and this is also supported by the increase in the rate of nutrient uptake by that culture. However, the additional nutrients may have

TABLE 2

ALGAL GROWTH AND NUTRIENT COMPOSITION

Biological System	Initial Wet Weight grams	Final Wet Weight grams	Initial Dry Weight grams	Final Dry Weight grams	Per Cent Increase in Dry Weight	Per Cent N in Dry Weight of Algal Growth	Per Cent P in Dry Weight of Algal Growth
Unsterile	10.100	10.430	.505	.521	3.2	9.0	2.5
Sterile	9.350	9.550	.470	.480	2.1	12.0	3.6

been utilized by bacterial cells or phytoplankton present in the unsterilized solution. Since there was no visual evidence of any large phytoplankton present and since several algae have been established as living in a state of symbiosis with bacteria (9), it is conceivable that the algal growth could have been influenced by the bacteria present. Because the variations in nutrient uptake of biomass production are slight enough to possibly be accounted for by experimental error, additional experiments are necessary before any definite conclusion can be stated on such possibilities as symbiotic relationships.

Nutrient limitation has been a subject of much concern because of the rise in nuisance algal growth resulting from eutrophication. A first step in controlling algal growth is the determination of the most probable limiting element; carbon, nitrogen and phosphorus are the most likely elements to be available in limiting quantities.

Because of the high stoichiometric ratio of carbon to other elements utilized by algae (10), carbon must be considered a limiting possibility. Since the atmosphere is one of the main sources of carbon dioxide in natural waters, the limiting probability of carbon in the Eau Gallie River may be approximated by comparing the atmospheric input and algal uptake rates of carbon. Although the rate of entry of carbon dioxide into water will vary depending upon the temperature, turbulence and the partial pressure of carbon dioxide in the atmosphere, an average intake rate of 0.177 $\mu g/cm^2$/minute has been estimated for the ocean as a whole (11). Since the average turbidity of the Eau Gallie River is 60 J.U. (Jackson Units), the average depth of the photic zone is about 34 cm (12). Assuming a depth of 34 cm and the above rate of carbon dioxide influx, carbon would enter river water at a rate of 177 µg-atom/day/liter.

RESULTS AND DISCUSSION

Several dissimilarities were found in the nitrate assimilation curves of the two culture media tested. On the initial day of monitoring, the unsterilized culture absorbed 150 ppb N/nitrate while the nitrate concentration in the sterile solution remained constant. The average N/nitrate uptake per day for the unsterile culture was 150 ppb corresponding with only 100 ppb for the sterile solution. One exception to the greater N/nitrate uptake by the unsterile solution was the negligible assimilation recorded on the fourth day.

The daily phosphate assimilation for both cultures was quite similar. Substantial and equivalent phosphate absorption occurred during the first day for both cultures and was twice as great as the next largest recorded decrease. This observation is consistent with experimentation of many planktonic algae which have the ability to store phosphate beyond immediate nutritive requirements when excess quantities are available (7). Successive phosphate increments were much more gradual. As observed with nitrate, the rate of assimilation of phosphate by the unsterilized culture was also greater throughout the experiment than by the sterile culture.

A gradual pH rise was observed in both test media, but the large pH variations of the unsterilized water on the fourth and fifth days of experimentation are not understood. It is noted that the large pH decrease on the fourth day was accompanied by a negligible nitrate uptake. Possible reasons for this large vacillation in pH include variation in photosynthetic activity and/or bacterial activity. In addition, the existence of other ions in solution as well as gases which entered the sample containers from the surrounding air could have been of considerable consequence since no attempt was made to measure or control these external forces (8).

By assuming that the atomic ratios of carbon to nitrogen and/or phosphorus in algae are equal to the ratio of their respective rates of algal uptake, and by utilizing the rates of phosphorus and nitrogen consumption by a typical mass of algae in the Eau Gallie River (Table 3), the algal uptake rate of carbon was determined and is recorded in Table 4.

TABLE 3
AVERAGE UTILIZATION RATES AND AVAILABILITY OF NITROGEN AND PHOSPHORUS IN THE ALGAE EXPERIMENT

Element	Average Availability Per Culture (μg - at/l)	Average Ratio of Availabilities	Average Uptake Rate μg - at/l day	Average Ratio of Availability To Uptake	Average Time Until Depletion (day)
P	6.1	1	.85	1.2	7.2
N	119.6	19.6	7.8	2.5	15.4

TABLE 4
CARBON UPTAKE RATES

Atomic Ratio (Used in Calculations)	Rate of Uptake of Carbon by Planktonic Algae μg - at/day	Rate of Uptake of Carbon by Freshwater Algae, Chlorella spp (μg - at/day)
C:N	55.0	69.6
C:P	95.4	42.3

By comparison of the rate of carbon inflow from the atmosphere (177 μg-at/day) to the calculated rates of carbon uptake by algae, it can be seen that carbon input rate from the atmosphere should normally exceed algal demand. In addition, the amount of carbon present is increased from a variety of sources such as organism respiration, bacteria or fungal decomposition of organic matter, sewage (untreated or treated), and land drainage (10). It is concluded that carbon is not likely to be present in limiting quantities in the Eau Gallie River.

From Table 3 it can be seen that the ratio of availability to utilization of nitrogen in the algae experiment was over twice as great as that for phosphorus and it may be calculated that after the phosphorus is depleted, 56.2 μg-at/1 of nitrogen is still available. Thus, it is concluded that phosphorus is the most probable limiting element in controlling algal growth in the Eau Gallie River.

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