# Phosphorus, Nitrogen, and Algae in Lake Washington after Diversion of Sewage

Abstract. *After diversion of sewage effluent from Lake Washington, winter concentrations of phosphate and nitrate decreased at different rates. From 1963 to 1969, phosphate decreased to 28 percent of the 1963 concentration, but nitrate remained at more than 80 percent of the 1963 value. Free carbon dioxide and alkalinity remained relatively high. The amount of phytoplanktonic chlorophyll in the summer was very closely related to the mean winter concentration of phosphate, but not to that of nitrate or carbon dioxide.*

Lake Washington has responded promptly to major changes in its nutrient income. In 1955 the lake was unmistakably deteriorating, for in that year *Oscillatoria rubescens* became prominent in the plankton (*1*). The lake was receiving effluent from ten secondary biological treatment plants amounting to about 24,200 $m^3$ [6.4 million gallons (1 gallon = 3.8 liters)] per day from a tributary population of about 68,000. In 1957, sewage effluent was contributing about 56 percent of the phosphorus and 12 percent of the nitrogen income of the lake (*2*). A program of diversion was voted by public action. Because of the magnitude of the project, diversion of all plants was not simultaneous. The first diversion of about one quarter of the effluent occurred in February 1963, by which time 11 treatment plants were enriching the lake with a total capacity of about 75,600 $m^3$ per day. In 1965 the volume was reduced to 55 percent of the original, and the final diversion took place in February 1968, but about 99 percent of it had been diverted by March 1967 (*3*).

The purpose of this report is to describe some of the changes in chemical conditions and algae following diversion of sewage effluent.

After the first diversion the condition began to improve steadily as indicated by the phosphorus content, abundance of algae, and transparency of the lake. In Lake Washington, concentrations of phosphate and nitrate are at a maximum in winter and decrease to low values during the spring growth of phytoplankton (*2*). During the winter of 1969, the concentration of phosphate was only 28 percent of that in 1963, the year of maximum phosphate. The content of nitrate has decreased much less than that of phosphate, remaining 80 percent or more of the 1963 value. The winter mean free carbon dioxide has been rather variable, fluctuating around 75 percent of the 1963 value. The winter mean alkalinity (bicarbonate) increased by about 20 percent from 1963. At present, an explanation for the details of the changes in carbon dioxide and alkalinity is not available.

During the years of heaviest enrichment, the number of phytoplankton has fluctuated around relatively high values during the summer (*4*), and the phosphorus content of particulate matter (seston) in summer corresponds well to the concentration of dissolved phosphate the previous winter (*5*). The abundance of phytoplankton has decreased since diversion started. The nuisance condition of a lake is well measured by Secchi disk transparency; the mean summer transparency has increased from 1.0 m in 1963 to 2.8 m in 1969. In Lake Washington, changes in transparency are dominated by changes in phytoplankton rather than in silt. Phytoplankton counts have not been completed, but data are available on the chlorophyll content of the phytoplankton in the epilimnion. The summer mean (July and August) is strikingly related to the concentration of phosphate in the surface water during the previous winter but not to nitrate or carbon dioxide or alkalinity (Fig. 1). This relation strongly suggests that phosphorus is the most important limiting element in Lake Washington. This, of course, does not mean that other elements are not important to phytoplankton, but only that they are present in excess relative to phosphorus, and the abundance of algae, therefore, varies in proportion to the phosphorus content. While it is conceivable that some other element, not measured in our study, is varying in exact proportion to phosphorus, it seems very unlikely.

The difference in the rates of decrease of nitrogen and phosphorus can be attributed at least in part to the fact that the water supply to the lake through the two major inlets is relatively much richer in nitrogen than in phosphorus, so that the lake water since 1963 has been progressively diluted with water that is poorer in phosphorus relative to nitrogen than is the water in the lake (*2*). Whether nitrogen fixation by blue-green algae has contributed to keeping the lake nitrogen elevated has not yet been determined.

The question has frequently been raised as to the effect of removal of phosphate from sewage effluent or of reducing the concentration of phos-

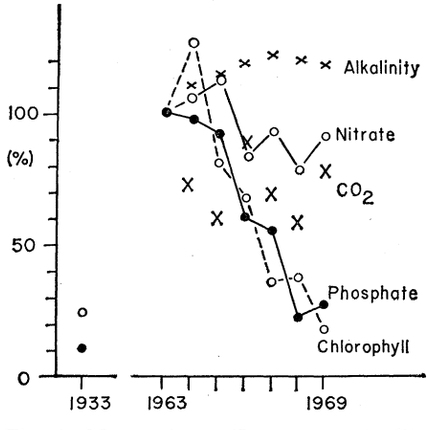


Fig. 1. Mean winter (January to April) values in surface water of phosphate-phosphorus and nitrate-nitrogen, and mean summer (July and August) values of chlorophyll in surface phytoplankton. The 1963 values, plotted as 100 percent, were (in micrograms per liter): P, 57; N, 428; and chlorophyll, 38. Unconnected points show winter means (January and February) of bicarbonate alkalinity and free carbon dioxide in surface water (25.3 and 3.2 mg/liter in 1963).

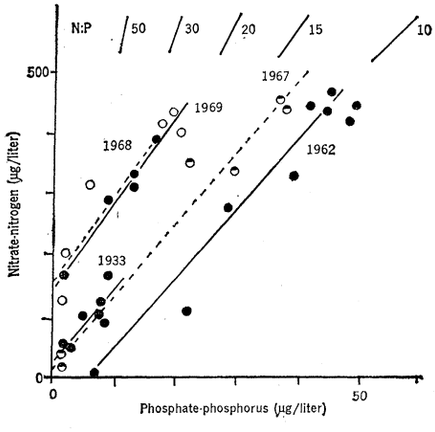


Fig. 2. Correlation between surface values of phosphate and nitrate during the spring increase of phytoplankton when the concentrations of nutrients are decreasing. In 1962 a distinct excess of phosphate occurred when nitrate had been exhausted, but in the other years shown nitrate was in excess, especially 1968 and 1969. The slopes of the lines, fitted by eye, vary from 11.9 (1962) to 14.8 (1968 and 1969). Ratios of nitrogen to phosphorus are shown by the numbered lines radiating from the origin. [Compare with fig. 5 of (*2*).]

phate in the effluent by controlling the character of the input. Concern has been expressed that heavy enrichment with nitrate may cause alga problems in waters with adequate natural supplies of phosphorus (*5*). While this might be true in some regions for geochemical reasons, it appears that Lake Washington in its unpolluted condition has more than enough nitrogen relative to phosphorus, and phosphorus is the dominating limiting element. Lake Washington probably represents a large class of lakes in which phosphorus is the dominating element (*6*).

An indication of the relative importance of these two elements can be seen by measuring their decreasing concentrations during the growth of phytoplankton in the spring (Fig. 2). In 1933, when the lake was less polluted, a small concentration of nitrate was left over when phosphate was nearly exhausted. In strong contrast, after many years of enrichment with sewage effluent rich in phosphorus, an excess of phosphate occurred when nitrate was exhausted in 1962; this excess phosphate was almost as much as the winter maximum of 1933. During diverison of sewage, the condition returned to resemble that of 1933 but continued to change, and in 1968 and 1969 a large excess of nitrate occurred. This kind of analysis should be generally useful in other lakes that have a winter maximum and spring decrease in nutrient concentration. It is obvious that the fact that phosphorus is in excess in a lake does not mean that control of phosphorus will be ineffective as long as it can be brought to low concentrations. Possibly in some regions excess phosphorus demonstrated this way can be regarded as evidence that the lake has been affected by sewage effluent and its quantity used as a measure of the magnitude of the effect.

Thus, Lake Washington has responded promptly and sensitively to changes in its nutrient income. While the changes during increase are not as well documented by direct limnological data as those during decrease, they are recorded by paleolimnological evidence (*7*). On the basis of the present data and existing knowledge, it seems valid to predict that noticeable improvements can be made in similar lakes even by partial limitation of phosphorus. Total elimination of phosphorus is impractical, of course. The worst sources are the most concentrated ones. The popular system of calculating total loadings on an areal basis (kilograms per hectare) without accounting for concentration can be misleading when dealing with lakes that receive large volumes of dilute drainage. The annual total of phosphorus can be very large, but if it is dispersed in a large volume of water it cannot generate as dense concentrations of algae as can the same total in much more concentrated sewage effluents and some kinds of agricultural drainage. Diversion of sewage from Lake Washington reduced the phosphorus income by only about half, but the sewage effluent was nearly 200 times as concentrated in phosphate as the influent streams (*2*), and the effect has been great.

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**References and Notes**

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8. Supported by grants from the National Science Foundation. Most of the field and chemical work in recent years has been carried out under the supervision of David E. Allison. Many of the data reported here have been obtained with the cooperation of Shirley M. Clark, Diane Egan, Donald J. Hall, Lois Kiehl, and Michael Parker.

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## Bone Marrow Colonies: Stimulation in vitro by Supernatant from Incubated Human Blood Cells

Abstract. *A substance which stimulates growth of granulocytic and mononuclear cell colonies from mouse and human bone marrow was produced by incubated human blood cells. It is resistant to heat and freezing and is not dialyzable. Intact irradiated and unirradiated cells had very little activity, and sonically disrupted cells had no activity. The addition of plasma or sonically disrupted cells to the cell supernatant decreased its activity.*

Bone marrow cells of animals (*1*) and man (*2–4*) can give rise to granulocytic and mononuclear cell colonies when grown in vitro in a soft gel medium with proper stimulation. A substance which stimulates the growth of colonies from murine marrow has been demonstrated in various cell feeder layers (*1*), certain serums (*5*), urine (*6*), and conditioned media obtained from various tissues (*7*, *8*). Feeder layers of kidney tubules (*4*), peripheral blood cells (*2*), and urine (*3*) are capable of stimulating colony growth from human bone marrow. The fact that a feeder layer of peripheral white blood cells will stimulate growth of marrow cells prompted our search for a substance which was either produced by or stored within circulating white blood cells.

Blood was collected from healthy adults with no known illnesses and prevented from coagulating with heparin. After sedimentation, the cell-rich plasma containing erythrocytes and leukocytes in a ratio of approximately 1 : 1 was incubated in McCoy's 5A medium containing fetal calf serum. The concentration of peripheral leukocytes was $2 \times 10^6$ to $4 \times 10^6$ cells per milliliter of medium. After 10 to 14 days of incubation at 37°C in 6.5 percent $CO_2$, cells were removed by centrifugation at 2000*g*, and the supernatant was passed through a filter with a pore size of 0.20 $\mu$m (Nalge Company, Rochester, New York). The ability of the supernatant to stimulate colony growth was tested by an adaptation of the soft-agar technique for cloning cells in vitro (*1*, *9*). Methylcellulose (Dow Methocel), as described by Ichikawa *et al.* (*10*) and modified by Worton, McCulloch, and Till (*8*), was used in place of agar. Bone marrow cells were removed from the marrow of 6- to 8-week-old $F_1$ mice from a mating of C57 BL females and DBA males. The cells were dispersed in CMRL-1066 and counted electronically (Coulter Electronics, Hialeah, Florida). A known number of cells was suspended in 1.4 percent methylcellulose containing tissue culture medium (CMRL-1066) plus 10 percent horse serum; for each 1 ml of mixture, 0.1 ml of the test substance was added. One milliliter of the mixture was then plated in 35 by 10 mm petri dishes (Falcon Plastics, Los Angeles, California) and incubated at 37°C in 6.5 percent $CO_2$. After 7 days, the number of colonies of cells growing within