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Algal Growth Stimulus by Phosphorus in Flathead Lake, Montana, Sediments

Abstract

Sediments from oligotrophic Flathead Lake stimulated more than double the growth of the algae *Selenastrum capricornutum* relative to control cultures in laboratory bioassays. Inorganic phosphorus, as well as other nutrients (e.g., nitrogen) in sediments were available as algal nutrients. These assays suggested that nutrients associated with sediments would likely stimulate algal growth in Flathead Lake. This growth apparently does not occur, because sediments derived from riverine sources have a short retention time in the photic zone of the lake. By inference we concluded that clay sediments entering the lake apparently play a functional role in sedimentation of phosphorus and other nutrients permanently to the lake bottom.

Introduction

Sediments may play a critical role in determining the trophic status of lakes, and a knowledge of the role of clay sediments is a concern in effective lake management (Serruya, 1971; Fillos and Swanson, 1975). Stanford and Potter (1976) suggested that the influx of clays during spring runoff influences the dynamics of the primary trophic level in Flathead Lake, Montana. The paucity of available phosphorus in this lake, 4-15 $\mu\text{g l}^{-1}$ (Stanford and Potter, 1976), probably makes this macronutrient an important one in directly influencing primary productivity.

Bottom sediments usually contain proportionately higher concentrations of nutrients than those found in the overlying water column due to gravitational losses of particulates (i.e., seston) and chemical precipitation. Consequently, sediments have long been recognized as a potential source of these nutrients to lake waters (e.g., Mortimer, 1941; Wetzel, 1975). However, little definitive information on the rate and extent of nutrient release from bottom sediments is available. The exchange of phosphorus between sediments and the overlying water appears to be dependent upon a number of chemical, physical, and biological parameters (cf. Lee, 1970; Wetzel, 1975). Laboratory studies (Golterman *et al.*, 1969; Porcella *et al.*, 1970) have shown that sediments may serve as the sole source of phosphorus to algae. Recent studies have attempted to quantify the different forms of sediment phosphorus and their potential as a macronutrient (cf. Wildung and Schmidt, 1973; Sagher *et al.*, 1975).

We performed laboratory bioassays to determine the biological availability of phosphorus in Flathead Lake sediments to a test alga, *Selenastrum capricornutum*. These experiments were a part of an ongoing study of the limnology of the Flathead Lake-River system to better define the functional role that clays may play in nutrient cycles within the lake.

Methods and Materials

Stock cultures of *Selenastrum capricornutum* were grown in synthetic assay medium mixed according to the *Selenastrum capricornutum* Prinz algal assay bottle test (U.S. EPA, 1978). In order to acclimate the alga, several successive cultures were grown in the AAP medium under the conditions of the assay. Since growth response relative to phosphate was to be examined, the alga was phosphorus-starved by growth under minus-phosphorus conditions. The medium used was AAP medium without the addition of the KH_2PO_4 ; KCl was used to compensate for the potassium ion concentration. The alga was allowed to grow in AAP minus P medium (AAP-P) to maximum biomass in 50 ml. To ensure phosphorus limitation to growth, cells were then harvested by centrifugation, washed with distilled water, re-centrifuged, and transferred to fresh AAP minus P medium. The process was repeated until growth was less than 10 percent of controls over a 24-hr period. The P-starved *Selenastrum* were then used as inoculum for the assay.

A coring device was used to collect sediments in the deepest area of Flathead Lake (100 m) at a point near midlake and west of Yellow Bay. Sediment cores were kept frozen until used in bioassays. The top 5 mm of core was homogenized, dried at 105°C for 72 hr, crushed, and added to the assay media at a concentration of 1 gm l^{-1} .

The effect of sediments free of inorganic P upon algal growth was determined by using sediments treated with an extraction procedure employing high temperature ignition (Walker and Adams, 1958). This procedure was tested and evaluated against other commonly used methods (Jackson, 1955; Mehta *et al.* 1958) for the extraction of inorganic phosphorus from sediments and was judged to be the most suitable (Wildung and Schmidt, 1973). The method called for adding 100 ml of 1N N_2SO_4 gm^{-1} of dried sediment followed by shaking for 16 hr. The sediment was then centrifuged, washed, centrifuged again, and then neutralized with NaOH. After drying and crushing, extracted and unextracted sediments were used in the assay. Of interest was the hypothesis that added sediment-phosphorus would significantly stimulate algal growth in comparison to controls in P-limited cultures. The bioassay was not, however, designed to differentiate between possible sources of inorganic phosphorus in the sediment, or their relative contributions to algal nutrition.

Six sets of three 50 ml replicates of the two experimental media (AAP and AAP-P) were inoculated with equal volumes of phosphorus-starved algae from the stock culture. Each culture set was then treated with extracted or unextracted sediments or untreated (controls) according to the experimental design shown in Table 1. An electronic particle counter (Coulter, Model ZBI) was used to determine cell numbers every 24 hr. Several cell counts were also made using standard microscopic procedures to ensure accuracy. Daily culture biomass (cell concentration) was calculated as an

TABLE 1. Experimental design and bioassay results; data are mean cell concentrations (cells $\text{ml}^{-1} \times 10^5$) ± 1 standard deviation ($n = 3$) at the conclusion of the bioassay (8 days).

Treatment	Medium	
	AAP-P	AAP
Unextracted sediment	50.4 \pm 12.8	64.0 \pm 2.0
Extracted sediment	7.2 \pm 0.4	46.9 \pm 7.2
Controls (no sediment added)	5.0 \pm 0.4	26.2 \pm 2.3

average of cell concentrations in each of the replicates within a given treatment. Significant difference between means was determined using a paired t-test ($\alpha = 0.05$).

Our bioassays examined only the biological availability of phosphorus, since not all forms of extractable inorganic phosphorus are available for algal nutrition. Some forms (i.e., apatite mineral, a form of occluded inorganic phosphorus) are not readily exchangeable (Li *et al.*, 1972) and were not considered to be a contributor to the dissolved phosphorus pool. These bioassays were designed to determine if inorganic phosphorus present in any form was available for algal growth under the test conditions.

Results and Discussion

The assays continued for 11 days; however, by day 9 all cultures had approached asymptotic growth. Cultures containing phosphorus (AAP medium) responded with growth that was significantly greater than observed in control cultures containing no phosphorus (AAP-P medium) (Table 1). Cultures in AAP-P medium containing extracted sediment were not significantly different from the AAP-P control cultures, which indicated that inorganic phosphorus had been removed by the extraction procedure (Table 1). Cell biomass in AAP control cultures was significantly less than cell biomass in the three other culture conditions (i.e., AAP plus extracted sediment, AAP plus sediment and AAP-P plus sediment) (Table 1). When sediments were added at the experimental concentration to AAP-P medium, final cell biomass was nearly twice that of the control cultures (Fig. 1). We concluded that sediment-phosphorus was available in sufficient quantity to stimulate a significant growth response.

We also concluded that other nutrients (e.g., nitrogen) were available in the sediments and that they apparently enhanced the stimulatory effect of phosphorus enrichment. The AAP cultures (containing $0.186 \text{ mg l}^{-1} \text{ PO}_4$) treated with extracted sediments yielded greater biomass than AAP controls. Since significantly greater growth stimulus was not manifested in AAP-P cultures treated with extracted sediments in comparison to AAP-P controls, it was apparent that growth in AAP cultures was stimulated by nutrients other than phosphorus in sediments (Table 1).

Microscopic examination revealed that bacteria were present during the assays. Bacterial remineralization of particulate organic matter in the sediments may have been a factor affecting phosphorus availability to test algae. However, the within treatment standard deviations were not particularly great, indicating that bacterial remineralization and other bias effects were fairly uniform across the design.

Turbidity caused by sediment addition may have introduced a difference in light availability between control and treated cultures; this factor made direct comparisons of growth response to nutrient concentrations difficult. Small responses due to minor nutrient limitations may be difficult to detect quantitatively in such assays, unless differential illumination effects caused by sediment turbidity can be measured. However, available inorganic phosphorus concentrations were great enough in these experiments to offset potential light availability effects, and they resulted in clear growth stimuli.

The deep basin (mean depth = 50.2 m) of Flathead Lake restricts sediment resuspension, except in highly localized near-shore areas. Also, the water column is usually saturated with oxygen; anaerobic conditions, which may facilitate phosphorus release from the sediments (Mortimer, 1941; Lee, 1970), do not occur. These limnological conditions and the ability of the bottom sediments to supply phosphorus in the bio-

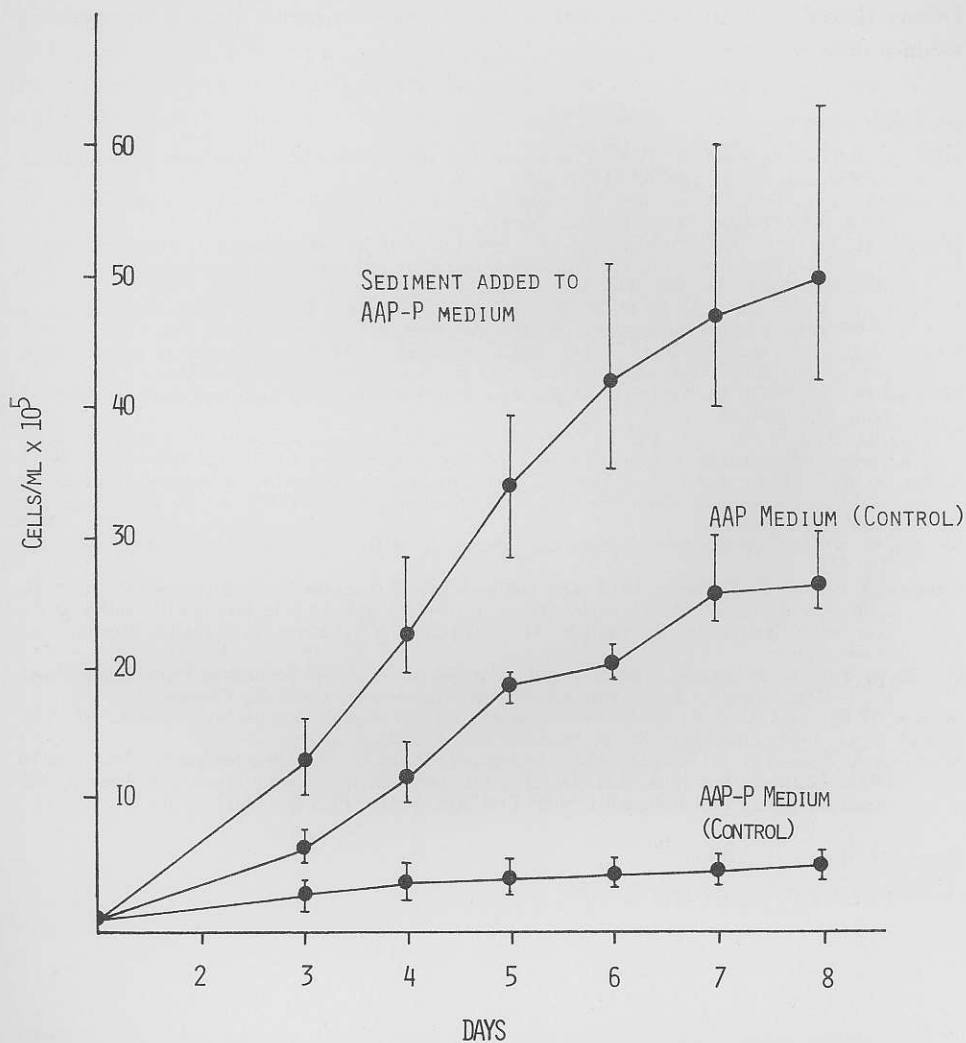


Figure 1. Growth of *Selenastrum* (means and ranges) in P-limited (AAP-P) and normal (AAP) control cultures in comparison to P-limited cultures treated with unextracted sediments from Flathead Lake.

assay implied that Flathead Lake sediments may expedite the permanent loss of this macronutrient from nutrient cycles in the lake. The low concentrations of phosphorus in the water and the higher concentrations of phosphorus in the profundal sediments add support to the hypothesis (Stanford and Potter, 1976) that sedimenting clays introduced during spring runoff may play a role in limiting primary productivity.

Acknowledgments

The bioassays were completed in the Limnology Laboratory at North Texas State University, Denton, Texas. Financial and material support was provided by the NTSU Faculty Research Committee and University of Montana Biological Station. We thank

Delano Hanzel, Montana Department of Fish, Game and Parks, for help in obtaining sediment cores.

Literature Cited

- Fillos, J., and W. R. Swanson. 1975. The release rate of nutrients from river and lake sediments. *J. Wat. Poll. Contr. Fed.* 47: 1032-1042.
- Golterman, H. L., C. C. Badels, and J. Jakobs-Mogelin. 1969. Availability of mud phosphates for the growth of algae. *Verh. Internat. Verein. Limnol.* 17: 467-479.
- Jackson, M. L. 1958. *Soil Chemical Analysis*. Prentice-Hall Inc., Englewood Cliffs, New Jersey.
- Lee, G. F. 1970. Factors affecting the transfer of materials between water and sediments. *U. Wis. War. Res. Centr. Lit. Rev. No.* 1.
- Li, W. C., D. E. Armstrong, J. D. H. Williams, R. F. Harris, and J. K. Syers. 1972. Rate and extent of inorganic phosphorus exchange in lake sediments. *Soil Sci. Soc. Amer. Proc.* 36: 279-285.
- Mehta, N. C., J. O. Legg, C. A. I. Goring, and C. A. Black. 1954. Determination of organic phosphorus in solids: I. Extraction method. *Soil Sci. Soc. Amer. Proc.* 18: 443-449.
- Mortimer, C. H. 1941. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 29: 280-329.
- Porcella, D. B., J. S. Kumagai, and E. J. Middlebrooks. 1970. Biological effects on sediment-water nutrient interchange. *J. Sanitary Eng. Div., Prov. Amer. Soc. Civil Eng. SA4:* 911-926.
- Sagher, A., R. F. Harris, and D. E. Armstrong. 1975. Biological Availability of Sediment Phosphorus to Microorganisms. Water Resources Technical Report: OWRT A-040-WIS. Univ. of Wisconsin, Madison.
- Serruya, C. 1971. Lake Kinneret: the nutrient chemistry of the sediments. *Limnol. Oceanogr.* 16: 510-521.
- Stanford, J. A., and D. S. Potter. 1976. The Flathead Lake River ecosystem: a perspective, pp. 241-250 in Andrews, R. D. III, *et al.* (eds.) *Terrestrial and Aquatic Ecological Studies at the Northwest Symposium Proceedings*, March 26-27, 1976. Eastern Washington State College Press, Cheney.
- U.S. Environmental Protection Agency. 1978. The *Selenastrum capricornutum* Prinz Algal Assay Bottle Test. Corvallis Environmental Research Laboratory, Corvallis, Oregon.
- Walker, T. W., and A. F. R. Adams. 1958. Studies on soil organic matter. *Soil Sci.* 85: 307-318.
- Wetzel, R. G. 1975. *Limnology*. W. B. Saunders Co., Philadelphia, Pa.
- Wildung, R. E., and R. L. Schmidt. 1973. Phosphorus Release from Lake Sediments. Proj. 16010 DUA. National Eutrophication Research Program, Environmental Protection Agency, National Environmental Research Center. EPA Res. Series: EPA-R3-74-024.

Received March 20, 1980

Accepted for publication July 7, 1980