Response of Lake Superior algae to nutrients and taconite tailings

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A LAKE SUPERIOR nutrient spiking study was conducted from November 1971 to May 1973. The principal objectives of the 19-month study were to (*a*) determine the algal-limiting nutrients in Lake Superior, (*b*) determine whether the limiting nutrient changes with time, and (*c*) determine the stimulation potential of taconite tailings and the algal response to taconite tailings additions at different times of the year.

The influence of taconite tailings on algal productivity was investigated because Lake Superior is receiving daily additions of tailings [1-4] from the Reserve Mining Company. The initial rate of discharge in 1955 was 20,000 tons/day (18,140 metric tons/day), and the present discharge, based on the rated capacity of the plant, is approximately 60,000 tons/day (54,400 metric tons/day). The Reserve Mining Company has received permits to discharge their tailings in this manner.

PROCEDURE

Twenty-two samples were collected during the study. Fourteen samples were collected at the Reserve plant intake at Silver Bay, Minn., six samples were collected outside the Reserve harbor, one sample was collected near Marquette, Mich., and one sample was obtained from the Sault Ste. Marie, Mich., power canal. On sampling days, a 1- to 2-gal sample of Lake Superior water was placed in an acid-washed polyethylene bottle. The sample was kept in the dark and returned to the Water Chemistry Laboratory, University of Wisconsin, Madison, via commercial airlines, Greyhound package express, or car, depending on when and where the sample was collected. In most cases, sample processing was begun within 24 hr of collection.

The first step in processing the samples was to determine the initial productivity of the sample. Six 25-ml aliquots of the sample were spiked with 2.0 μc of $^{14}C\text{-}CO_3^{2-}$ and incubated for 4 hr. Four of the replicates were incubated under 400 ft-c (4,300 lumens/sq m) supplied by fluorescent lights, as suggested in the Provisional Algal Assay Procedure (PAAP) (now the Algal Assay Procedure).[5] The remaining two samples were incubated at the same temperature as the first four samples (20°C), but in the dark. After the 4-hr incubation period, the samples were filtered through 0.45-μ pore size Millipore filters at a pressure differential equivalent to 6 in. (15.2 cm) of mercury by using a vacuum pump. During the filtration step, the sample bottles were rinsed twice with distilled water, and the filters were rinsed five times with distilled water. The total wash volume was approximately 50 ml.

Filters were placed in a desiccator containing concentrated HCl for 0.5 hr and then in a second desiccator containing silica gel, copper sulfate, and calcium oxide for an additional 0.5-hr period. The dried filters were dissolved in 10 ml of scintillation cocktail and 5 ml of 4:1 dioxane plus water. Each sample was counted for 20 min to determine the amount of ^{14}C incorporation during the incubation period. Sample counts were corrected for counting time and quenching and reported as carbon fixation in counts per minute per 4 hr. Net carbon fixation for the sample was calculated by subtracting the average dark

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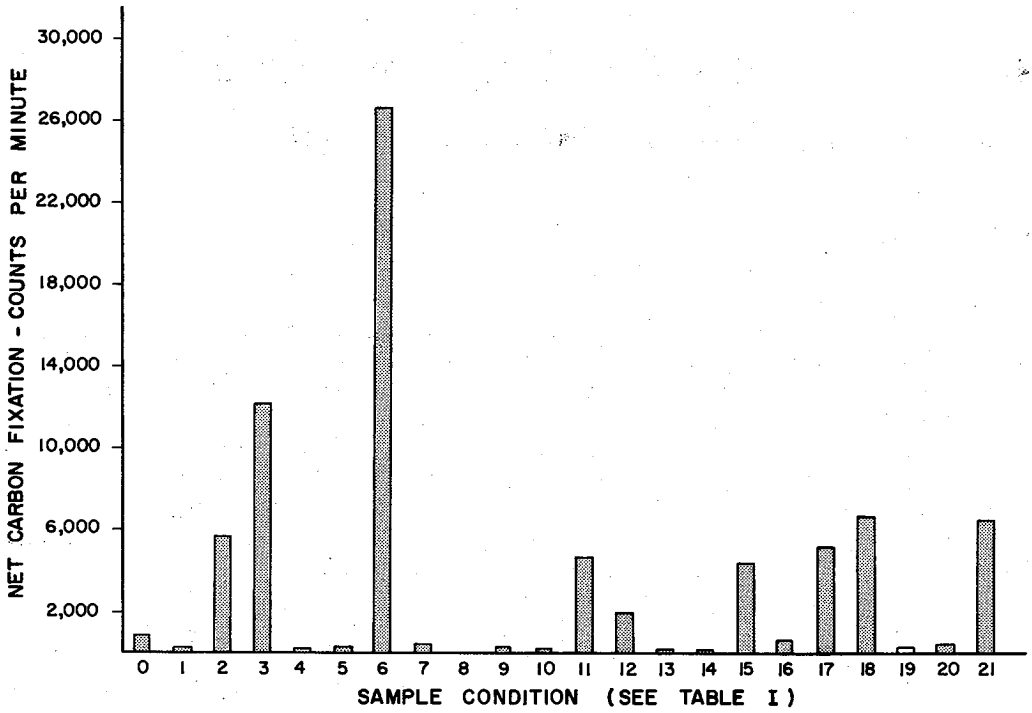

FIGURE 1.—Response of Lake Superior algae to nutrient and taconite tailings additions.

bottle fixation from the average light bottle fixation.

In addition to determining the initial productivity of the sample, the initial pH, temperature, and alkalinity were also recorded for the sample.

Once the initial productivity determination was in progress, the experiment was continued to determine the effect of nutrient additions. Working solutions of phosphate, nitrate, silica, manganese, PAAP medium, PAAP trace metal supplement, and taconite tailings were prepared by diluting stock solutions on the day they were needed. The concentration of these solutions was such that the addition of 1 ml working solution to a 25-ml lake water sample would result in the desired final concentration. The 25-ml aliquots of the initial lake water sample were placed in 50-ml culture flasks. The nutrient solutions and tailings suspensions were then added singularly and in combination to the lake water sample. Six replicates were prepared for each experimental condition that was established. The flasks were then sealed with foam plugs and incubated for approximately 2 wk at 20°C under 400 ft-c (4,300 lumens/sq m) of light.

Following the 2-wk incubation period, 2.0 μc of $^{14}C\text{-}CO_3^{2-}$ were added to each of the culture flasks, which were incubated for an additional 4-hr period. As discussed for the initial samples, four replicates from each set were incubated exposed to lights, and the remaining two samples were incubated in the dark. The short, 4-hr incubation period was completed under the same conditions of temperature and light intensity as the longer 2-wk period except for keeping the designated dark bottle samples out of the light. The samples were filtered, and the filters were processed as described earlier. The net carbon fixation was calculated as the difference between average light bottle fixation and average dark bottle fixation, and compared with initial and incubated lake water samples to

determine whether the experimental condition stimulated the Lake Superior algae.

REAGENTS

The following solutions or suspensions were used in the nutrient spiking study:

1. A stock phosphate solution was prepared by dissolving KH_2PO_4 in glass-distilled water.
2. The stock silica solution was prepared by dissolving sodium meta silicate in distilled water.
3. The manganese stock solution was prepared by dissolving Mn metal in concentrated HCl with glass-distilled water.
4. Nitrogen added to the Lake Superior cultures was in the form of nitrate. A stock solution was prepared by dissolving $NaNO_3$ in glass-distilled water.
5. A taconite tailings suspension was prepared by quiescent settling of total tailings in distilled water. The purpose was to obtain -2 μ-sized tailings for the study.
6. The final suspension contained 1.4 mg tailings/ml. Spiking a 25-ml Lake Superior water sample with 1 and 5 ml of the stock suspension resulted in tailings concentrations of 54 and 233 mg/l, respectively.
7. A working disodium ethylenediamine tetraacetate (EDTA) solution was prepared by diluting a stock Na_2 EDTA $\cdot 2H_2O$ solution prepared by dissolving the salt in glass-distilled water.

The PAAP medium was used as the algal medium in this study. This medium was prepared in four stock solutions. The first solution contained 17 g/l $NaNO_3$, 3.8 g/l $MgCl_2$, 9.8 g/l $MgSO_4 \cdot 7H_2O$, and 2.94 g/l $CuCl_2 \cdot 2H_2O$. The second solution contained 10 g/l Na_2CO_3, and the third contained 0.074 g/l $FeCl_3 \cdot 6H_2O$. The fourth solution was a trace metal supplement and contained 123.7 mg/l H_3BO_3, 176.2 mg/l Mn, 21.8 mg/l $ZnCl_2$, 0.52 mg/l $CoCl_2$, 0.006 mg/l $CuCl_2$, 4.84 mg/l $Na_2MoO_4 \cdot 2H_2O$, and 1.49 g/l Na_2 EDTA $\cdot 2H_2O$. The working PAAP solution was prepared by mixing 5 ml of solution one, 5 ml of solution two, 5 ml of solution three, 5 ml of solution of four, 1 ml of stock phosphate, 4 ml of stock silica, and 15 ml of glass-distilled water. One ml of this mixed solution was added to each of the designated PAAP flasks.

Solution four mentioned above is a concentrated PAAP trace metal solution. This solution was diluted from 5 to 40 ml with distilled water to prepare the trace metal solution. One ml of the working trace metal solution was added to 25 ml of lake water to prepare the trace metal replicates.

Carbon-14 was added to the samples as a basic carbonate solution. Sealed ampules of ^{14}C-bicarbonate were purchased and diluted to the appropriate volume to produce a 2-μc ^{14}C/ml solution. Sodium hydroxide solution was added to the ^{14}C-bicarbonate solution to raise the pH to 10.4 and shift the carbonate equilibrium to favor the CO_3^{2-} species and to prevent losses of ^{14}C-CO_2.

Sample radioactivity was determined by liquid scintillation counting with a scintillation spectrometer. All samples were counted for 20 min, and the activity of each sample was reported in counts per minute after correcting the data for counting time and quenching. The quenching efficiency was 65 to 70 percent for the majority of the samples. Additional details on the experimental procedure may be obtained from Plumb.[4]

RESULTS AND DISCUSSION

An example of the results obtained in the study is presented as a bar graph in Figure 1, and the experimental conditions are outlined in Table I. The sample under consideration was collected at the Reserve Mining Company plant intake on February 1, 1973, and the spiked samples were incubated from February 2 until February 19. An examination of Figure 1 reveals the stimulating influence of phosphate on a mixed Lake Superior algae population. Ten sets of cultures were spiked with phosphate, alone or in combination, of which seven produced stimulation compared with incubated lake water. None of the other established conditions produced a stimulating effect. These differences were

TABLE I.—Additions to Lake Superior Water Sample.

Sample No.	Sample Addition
0	Initial lake water
1	Incubated lake water
2	12.3 μg PO_4-P/l
3	23.7 μg PO_4-P/l
4	270 μg NO_3-N/l
5	2.2 mg SiO_2/l
6	PAAP medium
7	PAAP trace metal supplement
8	38 μg Mn/l
9	54 mg tailings/l
10	233 mg tailings/l
11	54 mg tailings/l + 12.3 μg PO_4-P/l
12	233 mg tailings/l + 12.3 μg PO_4-P/l
13	54 mg tailings/l + 38 mg Mn/l
14	233 mg tailings/l + 38 mg Mn/l
15	54 mg tailings/l + 38 mg Mn/l + 12.3 μg PO_4-P/l
16	233 mg tailings/l + 38 mg Mn/l + 12.3 μg PO_4-P/l
17	EDTA + 12.3 μg PO_4-P/l
18	38 μg Mn/l + 12.3 μg PO_4-P/l
19	EDTA
20	EDTA + 38 μg Mn/l
21	EDTA + 38 μg Mn/l + 12.3 μg PO_4-P/l

significant at the 90 percent confidence level based on the variation in the four light bottle replicates.

The data for the remaining sample sets are summarized in Tables II and III. Table II is the response of Lake Superior algae to the imposed spiking condition in comparison with the rates of carbon fixation in the initial sample, and Table III is the algal response to the spiking condition in comparison with the rates of carbon fixation in the incubated water sample. The data were analyzed at the 90 percent confidence level based on the variation in light bottle samples of each set.

Thirty-eight samples were spiked with phosphate only (Table II). Twenty-six of the phosphate-enriched cultures had a final rate of carbon fixation that was greater than the rate of carbon fixation in the initial lake water sample. A total of 143 samples were spiked with phosphate, either alone or in combination with other nutrients or taconite tailings. One hundred two samples were stimulated in relation to the initial lake water samples. This would suggest that phosphate is certainly a key limiting element in Lake Superior.

The possibility of manganese stimulation of Lake Superior algae was suggested by Shapiro [6] and was investigated as part

TABLE II.—Response of Incubated Samples to Initial Lake Water Samples.*

Experimental Conditions	Inhibition	No Effect	Stimulation
Incubated lake water	10	9	1
9.6 μg PO_4-P/l			
12.3 μg PO_4-P/l	1	5	12
16 μg PO_4-P/l			
23.7 μg PO_4-P/l	2	4	14
32 μg PO_4-P/l			
112 μg NO_3-N/l			
270 μg NO_3-N/l	9	10	1
336 μg NO_3-N/l			
520 μg NO_3-N/l	1		
2.2 mg SiO_2/l	12	8	1
PAAP medium	1	3	14
Trace metal supplement	6	10	1
23 μg Mn/l		1	
38 μg Mn/l	11	9	
46 μg Mn/l			
0.54 mg tailings/l		1	
5.4 mg tailings/l	5	1	
54 mg tailings/l	10	10	1
233 mg tailings/l	10	11	
EDTA	4	3	
12.3 μg PO_4-P/l + 270 μg NO_3-N/l		2	
12.3 μg PO_4-P/l + EDTA	1	2	11
12.3 μg PO_4-P/l + 38 μg Mn/l	1	2	5
38 μg Mn/l + EDTA	3	2	
5.4 mg tailings/l + 12.3 μg PO_4-P/l			2
54 mg tailings/l + 12.3 μg PO_4-P/l	1	2	16
233 mg tailings/l + 12.3 μg PO_4-P/l	1	1	11
5.4 mg tailings/l + 38 μg Mn/l	1	1	
54 mg tailings/l + 38 μg Mn/l	6	3	2
233 mg tailings/l + 38 μg Mn/l	7	4	
54 mg tailings/l + 38 μg Mn/l + 12.3 μg PO_4-P/l	2	3	5
233 mg tailings/l + 38 μg Mn/l + 12.3 μg PO_4-P/l	2	3	4
EDTA + 38 μg Mn/l + 12.3 μg PO_4-P/l	1	1	4
54 mg tailings/l + Azamine		2	
54 mg tailings/l + Azamine + 12.3 μg PO_4-P/l			2
233 mg tailings/l + Azamine	1	1	
233 mg tailings/l + Azamine + 12.3 μg PO_4-P/l			2

* At the 90 percent confidence level based on variation in four light bottles.

of this study by enriching 118 samples (including PAAP and trace metals) with manganese. Forty-one replicate cultures gave a negative response to manganese additions, 42 cultures showed no effect, and 35 cultures gave a positive response. These numbers include singular and combination additions of manganese. If those manganese cultures that also received phosphate are not considered, the remaining manganese-enriched cultures produced negative effects in 34 cultures, no effect in 30 cultures, and stimulation in three cultures. If one considers those cultures spiked only with manganese, none of the 21 samples was stimulated in relation to carbon fixation in the initial samples (Table II). It would seem that manganese is not a stimulatory agent for nearshore Lake Superior algae.

One objective of this study was to gather additional data on the nutrient potential of taconite tailings. One hundred thirty-four cultures were spiked with taconite tailings plus other nutrients, and 49 cultures received only tailings additions. The response in all tailings cultures was 46 negative, 43 the same, and 45 positive (Table II) in comparison with the initial rate of carbon fixation in the samples. As mentioned previously, these responses were the sum of all cultures receiving taconite tailings. A better idea of the algal response to taconite tailings is available from those cultures that were spiked with tailings only. There were 49 such replicate cultures in this study, and only one sample produced an apparent stimulatory effect. Among the remaining cultures, 23 showed no effect and 25 had negative responses. This strongly suggests that taconite tailings are nonstimulatory.

Tailings containing cultures were also broken down into phosphate-containing and phosphate-deficient cultures. Seventy-seven sets of replicate cultures with tailings did not have any added phosphate; the other 57 cultures were spiked with combinations of tailings and phosphate. Almost 95 percent (73 of 77) of the tailings cultures without phosphate were less than or equal to the initial lake water control. Stimulation was observed in 74 percent (42 of 57) of the Lake Superior cultures that were incubated with tailings and phosphate (Table II). Because tailings alone did not stimulate the Lake Superior algae population and because tailings plus phosphate

TABLE III.—Response of Incubated Samples to Incubated Lake Water Samples.*

Experimental Conditions	Inhibition	No Effect	Stimulation
9.6 μg PO_4-P/l		1	
12.3 μg PO_4-P/l	1	3	15
16 μg PO_4-P/l		1	
23.7 μg PO_4-P/l		5	15
32 μg PO_4-P/l			1
112 μg NO_3-N/l		1	
270 μg NO_3-N/l		19	
336 μg NO_3-N/l		1	
520 μg NO_3-N/l		1	
2.2 mg SiO_2/l	1	18	
PAAP medium		1	14
Trace metal supplement		14	4
23 μg Mn/l		1	
38 μg Mn/l	1	16	2
46 μg Mn/l		1	
0.54 mg tailings/l		1	
5.4 mg tailings/l	1	4	
54 mg tailings/l	3	15	1
233 mg tailings/l	2	16	1
EDTA		7	1
12.3 μg PO_4-P/l + 270 μg NO_3-N/l		2	
12.3 μg PO_4-P/l + EDTA		1	12
12.3 μg PO_4-P/l + 38 μg Mn/l		1	7
38 μg Mn/l + EDTA	1	5	
5.4 mg tailings/l + 12.3 μg PO_4-P/l			2
54 mg tailings/l + 12.3 μg PO_4-P/l		2	16
233 mg tailings/l + 12.3 μg PO_4-P/l		2	12
5.4 mg tailings/l + 38 μg Mn/l		3	
54 mg tailings/l + 38 μg Mn/l		10	2
233 mg tailings/l + 38 μg Mn/l		10	2
54 mg tailings/l + 38 μg Mn/l + 12.3 μg PO_4-P/l		1	9
233 μg tailings/l + 38 μg Mn/l + 12.3 μg PO_4-P/l		4	5
EDTA + 38 μg Mn/l + 12.3 μg PO_4-P/l		1	5
54 mg tailings/l + Azamine		2	
54 mg tailings/l + Azamine + 12.3 μg PO_4-P/l			2
233 mg tailings/l + Azamine	1	1	
233 mg tailings/l + Azamine + 12.3 μg PO_4-P/l		1	1

* At the 90 percent confidence level based on the variation in four light bottles.

did stimulate it, it must be concluded that taconite tailings are nontoxic.

In addition to studying the influence of phosphate, manganese, and taconite tailings on the algal population, the effect of nitrate, silica, and trace metals was studied. Twenty of 21 nitrate samples, 20 of 21 silica samples, and 16 of 17 trace metal samples had a final carbon fixation rate that was less than or equal to the carbon fixation rate in the initial water sample. These data support the conclusion that nitrate, silica, and trace metals are not the factors controlling the algal populations in Lake Superior.

The discussion of the data in Table II was based on the results of the incubated cultures in comparison with the activity associated with initial lake water samples. A comparison of incubated cultures results with those of incubated lake water samples without additions is presented in Table III. The only singular addition that continually caused a positive response was phosphate. At least one sample in every set was stimulated by phosphate additions, and, on six occasions, all cultures spiked with phosphate had an increased rate of carbon fixation. The six sampling dates were June 30, July 25, August 1, October 18, November 2, and December 5. Overall, 74 percent (31 of 42) of those water samples receiving only phosphate additions had an increased rate of carbon fixation; 81 percent (116 of 143) of all samples receiving phosphate in any combination had an increased rate of carbon fixation (Table III).

The strong trend of phosphate stimulation that was observed in this investigation is in good agreement with past studies on Lake Superior. The previously cited work by Shapiro [6] suggested that manganese might be limiting offshore plankton, but also indicated that phosphate was limiting both nearshore and offshore populations. Schelske *et al.*[7] also reached the conclusion that phosphorus limits phytoplankton growth and production in Lake Superior. This was based on an *in situ* study in which they observed a threefold increase in algal numbers as a result of adding 7.5 μg of PO_4-P/l to lake water. Phosphate levels in this investigation were 12 μg PO_4-P/l and 23 μg PO_4-P/l.

Manganese addition results did not suggest that manganese is a key limiting nutrient in Lake Superior. Manganese was added to 21 samples in the study, and 86 percent of the samples (18 of 21) had the same rate of carbon fixation as the lake water controls. Only two samples with manganese additions were stimulated.

Taconite tailings were not a stimulatory agent. Only two samples out of 44 sample sets spiked with tailings only had a greater rate of carbon fixation than did the incubated lake water controls. Thirty-six tailings suspensions had the same final activity as that of the lake water controls; six samples had a lower rate of fixation (Table III). A total of 132 cultures were prepared with taconite tailings in combination with other additions. Only 8 percent of all tailings suspensions without phosphate (6 of 75) resulted in a positive response, whereas 82 percent (47 of 57) of the tailings plus phosphate cultures resulted in a positive response (Table III). These results support the position that taconite tailings are nonstimulatory and nontoxic.

The other nutrient conditions established in this study with trace metals, silica, and nitrate demonstrate that these materials play a minor role in limiting algal populations in Lake Superior. Trace metal additions stimulated four of 18 cultures, silica stimulated zero of 19 cultures, and nitrate stimulated zero of 22 cultures in comparison with incubated lake water samples (Table III).

CONCLUSIONS

A 19-month nutrient spiking study was conducted with samples collected primarily in the western arm of Lake Superior; it was concluded that phosphate is a key limiting nutrient controlling the algal population in Lake Superior. Rates of carbon fixation in phosphate-enriched cultures were compared with rates of carbon fixation in initial and incubated lake water

samples because the rate did decrease during incubation. Approximately 70 to 80 percent of all samples receiving phosphate in the nutrient spiking study had a greater rate of carbon fixation at the 90 percent confidence level. Trace metals stimulated 20 percent of the samples, manganese stimulated 8 percent of the samples, and nitrate and silica were not stimulatory for Lake Superior algae. No seasonal fluctuations were observed in the algal responses to the nutrient spiking conditions.

Taconite tailings additions did not stimulate the Lake Superior algae used in this study. Less than 5 percent of the tailings suspensions produced a net carbon fixation that was greater than that of the lake water controls. This study demonstrated that Lake Superior algae would respond to phosphate additions, and the fact that algae responded to tailings plus phosphate and not to tailings alone suggests that tailings will not contribute to the eutrophication of the lake and that taconite tailings are nontoxic to algae.

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