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ACID RAIN STIMULATION OF LAKE MICHIGAN PHYTOPLANKTON GROWTH

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ABSTRACT. Three laboratory experiments demonstrated that additions of rainwater to epilimnetic lake water collected in southeastern Lake Michigan stimulated chlorophyll a *production more than did additions of reagent-grade water during incubations of 12 to 20 d. Chlorophyll* a *production did not begin until 3-5 d after the rain and lake water were mixed. The stimulation caused by additions of rain acidified to pH 3.0 was greater than that caused by additions of untreated rain (pH 4.0-4.5). Our results support the following hypotheses: (1) Acid rain stimulates the growth of phytoplankton in lake water; (2) phosphorus in rain appears to be the factor causing this stimulation. We conclude that acid rain may accelerate the growth of epilimnetic phytoplankton in Lake Michigan (and other similar lakes) during stratification when other sources of bioavailable phosphorus to the epilimnion are limited.*

ADDITIONAL INDEX WORDS: Phosphorus, Chlorophyll a*, eutrophication, algae.*

INTRODUCTION

Precipitation poses a potential threat to water quality in the upper Great Lakes because it is acidic and contains higher concentrations of pollutants, including phosphorus (P), than upper Great Lakes waters (Murphy and Doskey 1976, Eisenreich *et al.* 1977, Parker *et al.* 1981). Because the lakes are well buffered, the acidity of acid rain does not substantially affect the pH of Great Lakes waters, but acid rain could still affect phytoplankton dynamics in the Great Lakes. One study, based on ^{14}C uptake measurements to estimate phytoplankton growth, suggested that acid precipitation inhibited the growth of Great Lakes phytoplankton (Parker *et al.* 1981). However, short-term measurements of ^{14}C uptake may be an inaccurate indicator of phytoplankton growth (Lean and Pick 1981). A second possible effect of acid rain could be enhancement of phytoplankton growth through additions of nutrients during periods of nutrient limitation (Paerl 1985). In this note, we present preliminary results on how acid rain can affect the growth of P-starved Lake Michigan phytoplankton (over incubation periods of up to three weeks) and explore reasons for this effect.

MATERIALS AND METHODS

To determine potential effects of untreated and acidified rain on phytoplankton growth, we measured chlorophyll *a* (Chl *a*) production through time in mixtures of rain and low-P lake water in three different experiments (Table 1, Fig. 1). In each, rain was collected and added within a few days to epilimnetic water collected from southeastern Lake Michigan during late summer, a period when epilimnetic phytoplankton are normally limited by P and/or silica (Schelske and Stoermer 1971, Schelske 1975, Fahnenstiel and Scavia 1987).

TABLE 1. Conditions of phytoplankton-growth experiments in which rain or reagent grade water (RGW) was added to epilimnetic, offshore water from Lake Michigan.

Experiment, Date and Conditions	Duration (d)	Treatment Number	Treatment Description
I 4 August 1982	12		0.5 L treatment water + 1.5 L lake water
Temperature = 24°C		1	RGW (control)
Light:dark = 16 h:8 h		2	Acidified RGW (pH 2.9)
Light intensity = 100 μEin m^{-2} s^{-1}		3	Acidified RGW (pH 2.9) + 7 μg P L^{-1}
		4	Rain (pH 4.0)
		5	Acidified rain (pH 3.0)
II October 15, 1982	20		0.5 L treatment water + 1.5 L lake water
Temperature = 15°C		1	RGW (control)
Light:dark = 14 h:10 h		2	Acidified RGW (pH 3.0)
Light intensity = 100 μEin m^{-2} s^{-1}		3	Acidified RGW (pH 3.0) + 7 μg P L^{-1}
		4	Rain (pH 4.5)
		5	Acidified rain (pH 3.0)
III September 20, 1983	13		0.5 L treatment water + 3.5 L lake water
Temperature = 20°C		1	RGW (control)
Light:dark = 14 h:10 h		2	Acidified RGW (pH 3.7)
Light intensity = 100 μEin m^{-2} s^{-1}		3	Acidified RGW (pH 3.7) + 15 μg P L^{-1}
		4	Rain (pH 3.2)
		5	Rain (pH 3.2)
		6	Filtered rain (pH 3.2)

Soluble reactive P (SRP) was below or near the level of detection (0.5 μg/L) in the lake water used for each experiment. All containers and equipment contacting the samples during experiments were washed with 20% HCl and rinsed with reagent grade water (RGW; Milli-RO system, Millipore Corporation), of pH 5.6 to 6.0, that contained no measurable SRP. Rainwater for experiments I, II, and III was collected in Ann Arbor, Michigan, on 27 July 1982, 15 October 1982, and 16 September 1983, respectively. Samples were collected with a slanted, V-shaped platform consisting of two sheets of plywood covered with a single 1.22 × 2.44-m sheet of linear polyethylene (LPE) plastic 1.5 mm thick. Immediately before a rainfall, the collector was washed with 20% HCl, rinsed repeatedly with RGW, and mounted with an LPE collection vessel on the roof of the National Fisheries Center. After collection, rain was stored at 4°C in a covered LPE container for 8 d (experiment I), 1 d (experiment II), or 3 d (experiment III). Offshore water was collected from Lake Michigan for experiments I, II, and III on 3 August 1982, 13 October 1982, and 19 September 1983, respectively, at a depth of 4 m about 20 km west of Grand Haven, Michigan. Lake water was stored at *in situ* temperature (15–24°C) and light conditions until it was combined with rain or RGW in various treatments (Table 1).

In each experiment, lake water was diluted with RGW or rain water at a ratio of 3:1 (volumes lake water: volume diluent) (experiments I and II) or 7:1 (experiment III) to compare phytoplankton growth rates under the various treatments. Treatment 1 was a control diluted with P-free RGW. The experimental treatments are summarized in Table 1. In each experiment, treatments with P-free RGW and acidified (pH 3.0) P-free RGW were

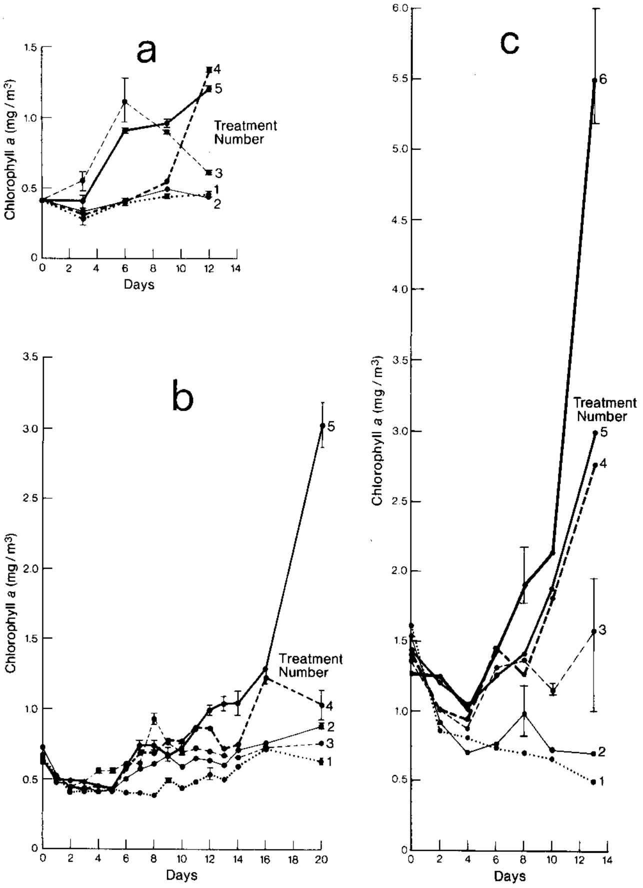

FIG. 1. Chl a *production vs. time in low-P epilimnetic Lake Michigan water in response to additions of reagent-grade water (RGW; treatment 1), acidifed RGW (treatment 2), RGW containing PO_4-P (treatment 3), unmodified rain (treatment 4), acidified rain (to pH 3; treatment 5), and filtered, unmodified rain of pH 3.2 (treatment 6, experiment III only). Each point is the mean of the indicated range of replicate Chl* a *measurements. Ranges not indicated were less than 10% of the mean. (a) Experiment I (August 1982); (b) Experiment II (October 1982); (c) Experiment III (September 1983). Because rain collected for experiment III had an initial pH of 3.2, it was not acidified further for treatment 5. Therefore, treatment 5 is a replicate of treatment 4 in experiment III.*

run as controls. In addition, acidified RGW was fortified with potassium phosphate to approximate the SRP content of rain. Untreated (pH 3.2–4.5) and acidified (pH 3.0) rain were added to lake water to examine the potential effects of normal and acidified rain on phytoplankton growth. The pH of the rain was not lowered in experiment III because the untreated rain for this experiment was already acidic (pH 3.2). Hence, treatments 4 and 5 were identical in this experiment. In experiment III, a portion of the untreated rain was filtered through a clean 0.2 μm pore size Nuclepore filter (treatment 6) before it was mixed with the lake water to examine the effect of removing particles from the rain on chlorophyll production. Temperatures and light:dark cycles approximating ambient lake conditions were used in all experiments (Table 1).

SRP was measured by the ascorbic acid method (Murphy and Riley 1962, Gardner and Malczyk 1983). For total P, the samples were first digested with heated potassium persulfate (Menzel and Corwin 1965). Chl *a*, corrected for phaeopigments (Strickland and Parsons 1972), was measured in triplicate water samples removed at intervals from the incubating vessels. Turnover times of ^{33}P were measured near the beginning (4 h after rainwater addition) and at the end of experiment III (Lean and Nalewajko 1979). The uptake rate constant was the log of the initial linear slope in the plot percent ^{33}P uptake vs. time (see Fig. 2). Turnover time (t), the reciprocal of the uptake constant, is an indicator of phytoplankton cell phosphorus-deficiency because it reflects the phosphorus status of the cell as well as biomass (Lean *et al.* 1983). Turnover times were calculated by the formula:

$$t\ (\text{min}) = 1/\ln(1 + S/100)$$

where S = initial linear slope (see Fig. 2).

To eliminate the effect of biomass on turnover time, all turnover times were multiplied by chlorophyll concentrations. With this correction, turnover times indicate the phosphorus status of the cells; long turnover times (large numbers) are indicators of relatively P-rich cells whereas short turnover times (small numbers) are indicative of relatively P-deficient cells.

RESULTS AND DISCUSSION

In all experiments, rain stimulated phytoplankton growth (Chl *a* production) relative to control treatments (Fig. 1). Measurements of pH in experiment I indicated that additions of acidified RGW or rain (pH 3.0) to lake water (initial pH 8.1) only slightly reduced the pH of the lake water (never below pH 7.0). Acid in RGW did not affect Chl *a* production, but acidified rain stimulated phytoplankton growth more than untreated rain (Fig. 1).

The observed enhancement by rain of phytoplankton growth in lake water differs from the results of Parker *et al.* (1981), who found no stim-

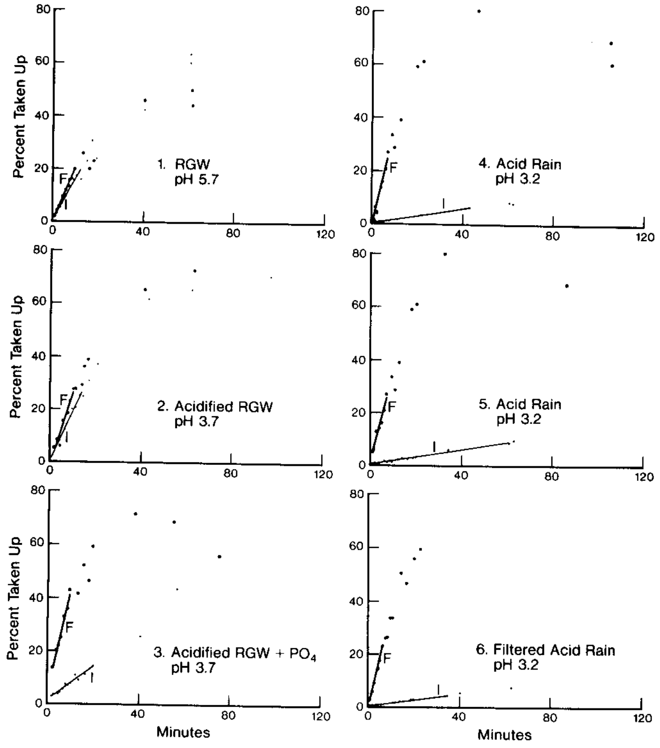

FIG. 2. Plots of ^{33}P uptake as percent taken up vs. time in initial (I) and final (F) ^{33}P measurements for each treatment at the beginning and end of experiment III. Regression lines with 95% confidence intervals for the initial slopes are indicated on each plot. The slope of each of these lines is the ^{33}P uptake constant.

ulation of ^{14}C uptake in short-term incubations when they added rain or snowmelt to water collected from Lake Michigan in April, May, July, and September 1979. This lack of agreement may stem from the fact that Parker *et al.* incubated their mixtures of rain and lake water for only a few hours rather than several days. In our experiments, phytoplankton growth response was not observed until 3 to 5 d after the experiments were begun (Fig. 1). This lag period was similar to that previously observed in nutrient enrichments of Lake Michigan phytoplankton (Schelske 1984).

In other studies (Lean and Pick 1981), ^{14}C uptake by P-deficient phytoplankton populations in lakes was depressed for the first few hours after a limiting nutrient was added. Results of previous nutrient enrichment experiments (discussed by Lean and Pick 1981) were consistent with the interpretation that when P is added, healthy P-deficient phytoplankton rapidly shift from fixing carbon to assimilating and storing P. This rapid consumption of P occurs within minutes or hours. In our experiments, added phosphorus disappeared rapidly but growth was delayed. Incubations of several days are apparently needed for significant growth to occur, a requirement for reliable determinations of nutrient limitation in natural waters.

In considering possible reasons for the enhancement of growth caused by addition of acid rain, we hypothesized that P, liberated in part by acidification, may have stimulated phytoplankton growth (Manny and Owens 1983). The results of experiments I and II (Figs. 1a and b) tentatively support the hypothesis that mild acid treatment may convert some form of P in rain to a more biologically available form. For example, in experiment I, Chl *a* concentration appeared to increase sooner in the acidified-rain (pH 3.0) than in the normal-rain (pH 4.5) treatment (Fig. 1a). In experiment II, differences in Chl *a* concentrations caused by acidification were not distinguishable during the first 12 days of the experiment, but the acidified-rain treatment produced more Chl *a* during the last 8 days than did the normal-rain treatment (Fig. 1b). In agreement with these tentative results for P, nitrogen from rain stimulated the growth of nitrogen-depleted marine phytoplankton exposed to acid rain (Paerl 1985). However, nitrogen is not a limiting nutrient for Lake Michigan phytoplankton, as nitrate is abundant in the lake (Bartone and Schelske 1982). Experiments in our laboratories indicated that acidification of rain can cause SRP to be chemically liberated from particles in the rain (Gardner and Manny, unpublished data). Assuming that P was the factor limiting phytoplankton growth in our experiments, these combined results suggest that acidification of rain entering the Great Lakes could potentially stimulate phytoplankton production by transforming P in the rain into a form available for rapid uptake.

Experiment III was designed to test the P-stimulation hypothesis. In addition to measuring SRP and Chl *a*, we quantified total P concentrations, and ^{33}P turnover times at the beginning and end of this experiment. Also, the ratio of diluent to lake water was smaller in experiment III (1:7) than in the first two experiments and phytoplankton were fresh from the lake when the experiment began.

SRP was removed from solution by the second day in all the treatments (data not shown), but, as would be expected from a mass balance, total P concentrations did not change substantially in any treatment over the course of the experiment

TABLE 2. Concentrations of P (SRP and TP; µg P L^{-1}) and turnover times of added ^{33}P [t, as measured (min), and t_c, after correction for phytoplankton biomass (mg Chl **a**. *min.m^{-3})] in treatments at the beginning and end of experiment III. Treatment numbers for experiment III are indicated in Table 1.*

	Initial				Final			
			^{33}P Turnover time				^{33}P Turnover time	
Treatment	SRP	TP	t	t_c	SRP	TP	t	t_c
1	0.8	3.0	62	98	<0.6	2.8	48	24
2	0.8	2.6	52	79	<0.6	2.5	38	26
3	5.5	4.7	159	216	<0.6	4.9	27	42
4	3.0	7.6	707	989	<0.6	6.8	27	74
5	2.0	7.9	700	1,012	<0.6	8.0	29	87
6	2.5	7.6	873	1,108	<0.6	8.1	27	149

(Table 2). The ^{33}P studies suggested that phosphorus was the factor limiting phytoplankton growth (Fig. 2). ^{33}P turnover rates, measured a few hours after rainwater addition, were slower (i.e., ^{33}P turnover times were longer) in treatments containing rain (treatments 4–6) than in treatments containing RGW (treatments 1 or 2) or PO_4-P in RGW (treatment 3). This result indicated that the phytoplankton receiving rainwater were more P-sufficient than were those in the other treatments (Fig. 2). If P was the element limiting phytoplankton growth, P-sufficient phytoplankton, at the beginning of the experiment, would be expected to grow more than P-deficient phytoplankton. In fact, final chlorophyll levels were significantly correlated ($r = 0.93$; $p < 0.01$) with initial turnover times (Fig. 3). However, at the end of experiment III, ^{33}P turnover times were short in all treatments (Fig. 2, Table 2) and indicated that the phytoplankton had taken up all available P and were again P-deficient. These results all suggest that phosphorus was likely the primary factor in the rain responsible for stimulating phytoplankton growth.

The addition of filtered rain to lake water (treatment 6, experiment III) caused higher Chl *a* production than did the addition of unfiltered rain (Fig. 1c). This result was not initially expected because particles in the rain were thought to be a potential source of P to phytoplankton. However, under acidic conditions, weakly associated P can be released from particles. For example, removal of fine, gray particles (5.6 mg L^{-1}) from the rain by filtration in treatment 6 did not measurably change the total P levels in the rain (Table 2). The initial ^{33}P turnover time was also slower in treatment 6 than in the treatments (4 and 5) with unfiltered rain, indicating that phytoplankton in treatment 6 were more P-sufficient than those in the other treatments. These results indicate that a pH-particle interaction may affect the availability of P from rain. Although P appears to be freed from particles by the acid in the low-pH rain, it may be partially resorbed onto the particles again when unfiltered rain comes into contact with the buffered lake water. This interesting preliminary result should be examined further to provide information about mechanisms controlling the potential availability of P from rain to phytoplankton. However, despite this potential competition between "rain particles" and phytoplankton for P, the net effect of adding rain (filtered or unfiltered) to lake water was to stimulate growth of P-deficient phytoplankton over that observed without rain.

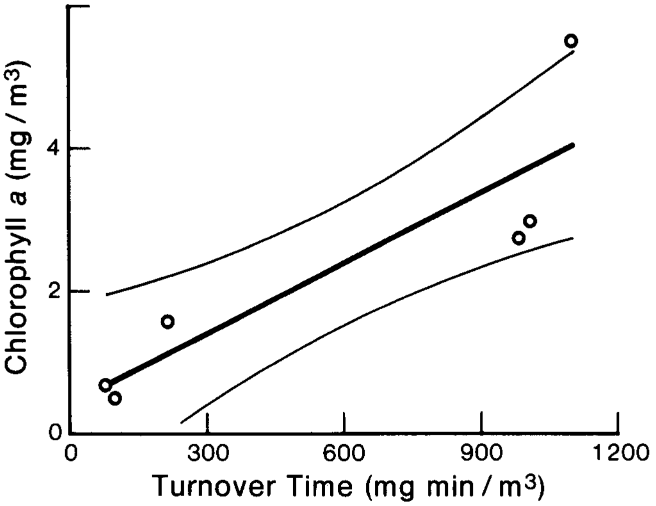

FIG. 3. Final Chl **a** *concentrations vs. biomass corrected ^{33}P turnover times for the various treatments in experiment III. $Y = 0.00301X + 0.339$, $r = 0.93$ ($p < 0.01$).*

The potential ecological effects of having P added to surface waters during a rainfall stems from the rapid consumption of available P by phytoplankton and the apparent sustenance of their growth for 1–2 weeks, even if the plankton do not remain in surface waters. Our data suggest that P from rain can stimulate the growth of P-limited phytoplankton and are consistent with the hypothesis that increased acidification may enhance the availability of P in the rain. These phenomena could be important to offshore epilimnetic phytoplankton production in the upper Great Lakes during periods of stratification when atmospheric input is likely the primary source of new bioavailable P to these organisms.

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