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Response of Periphyton Biomass to High Phosphorus Concentrations in Laboratory Experiments

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The use of periphyton as an efficient method to remove nutrients from water, and consequently to reduce eutrophication, was proposed by Sladeckova et al. (1983) and Vymazal (1988). However, in different environments algae show different responses to phosphorus enrichment. Margalef (1983) asserts that in waters with a pH 7 and 1 $\mu M.L^{-1}$ of calcium content, soluble reactive phosphorus (SRP) should not be higher than 0.3 $\mu M.L^{-1}$ as higher concentrations could produce eutrophication. Similarly, Horner et al. (1983) observed that more than 20 $\mu gP.L^{-1}$ (0.66 $\mu MP.L^{-1}$) were necessary to produce periphyton blooms. In constrast Bothwell (1985) observed blooms in waters with only 3-4 $\mu gP.L^{-1}$ (0.10-0.13 $\mu MP.L^{-1}$).

In some streams of the Buenos Aires Province, SRP concentrations are higher than the values reported by Bothwell (1985) and Horner et al. (1983) even upstream of urban and industrial effluents. Wetzel (1981) suggested that the approximate phosphorus load disposed of by a city is 2 $g.day^{-1}.inhabitant^{-1}$; therefore, concentrations in low discharge streams as those found in Buenos Aires (around 0.5 $m^{-3}.s^{-1}$) may be very high.

The aim of this study was to estimate, in laboratory experiments, the variation of periphyton biomass and taxonomical richness under the different phosphorus concentrations possible in plain streams of Buenos Aires Province.

MATERIALS AND METHODS

Periphyton of Typha latifolia (cattail) and water were taken with 5-L plastic bottles from the Gutierrez stream, a tributary of the Luján river (Buenos Aires Province, Argentina), which had high SRP levels (120 to 300 $\mu gP.L^{-1}$ during the entire year) (Giorgi et al. 1994). This stream, as most plain streams of Buenos Aires, has cattail beds on its course.

Three experiments were started in the Spring of 1991, when cattail development and periphyton colonization begin in natural environments of the region. Cattail stems collected in the Gutierrez stream were brushed and washed with filtered stream water in order to remove periphyton. After which, triplicate acetate sheets of 20 cm^{-2} with periphyton and without it (control) were used under differents treatments. They were put into 250-ml vessels filled with stream water filtered through a 30-µm pore diameter plankton net. Five mL of water with high concentrations of periphyton were dropped daily into each vessel over 15 days to allow colonization of the acetate sheets. Vessels were maintained at 25 °C (+/- 1°C) illuminated continuously by fluorescent light (approximately 2000 lux), and shaked daily. The experiments began after 15 days of development of the periphyton community. Chlorophyll-a values measured at that time were considered as the initial ones.

Algal biomass, quantified as chlorophyll-a, was used to estimate the community responses to phosphorus enrichment (Hanna and Dauta 1983).

In the first experiment, five different levels of phosphorus enrichment were tested: A) Without phosphorus addition; B) With addition of 1.5 mg $P\text{-}PO_4^{-}.L^{-1}$; C) 15 mg $P\text{-}PO_4^{-}.L^{-1}$; D) 150 mg $P\text{-}PO_4^{-}.L^{-1}$; and E) 1500 mg $P\text{-}PO_4^{-}.L^{-1}$ (Table 1a). After seven days, 50 mL of water were taken from all the vessels to estimate SRP by the ascorbic acid method (APHA 1975). At the same time, acetate sheets from the different treatments were removed to estimate chlorophyll-a concentration by the modified Lorenzen method (Aminot 1983). Algal richness (number of taxa) in each group was observed under a Nikon Optiphot microscope with 100X immersion objectives. Species abundance was estimated with a Braun-Blanquet scale with five degrees of covered area on the acetate sheets: **ha**, more than 80%; **a**, between 60-80%; **f**, between 40-60%; **e**, between 20-40% and **r**, between 5 and 20%. Taxa were quantified only in this experiment because it included all the ranges of P added.

In the second experiment, only two levels of phosphorus were used: A) With no phosphorus added; and B) with 1.5 mg $P\text{-}PO_4^{-}.L^{-1}$ added . Samples of water and acetate sheets were taken after 0, 3, 5, and 7 days to analyze SRP and clorophyll-a contents, respectively (Table 1b).

In the third experiment, three treatments were used: A) With no phosphorus added; Z) with addition of 750 mg $P\text{-}PO_4^{-}.L^{-1}$; and E) with addition of 1500 mg $P\text{-}PO_4^{-}.L^{-1}$. SRP content and clorophyll-a concentration of acetate sheets were analyzed after seven days (Table 1c).

Table 1. Experimental designs and mean soluble reactive phosphorus (SRP) levels before and after the experiments (in mgP-PO_4^{3-}.L^{-1}). Control: acetate sheets without periphyton. Colonized: acetate sheets with periphyton (in parenthesis: standard errors).

a) First Experiment
Sampling Period: 0 and 7 days.

Treatments	P Added	Final Values Control X	SD	Colonized X	SD
A	0	0.21	(0.01)	0.01	(0.01)
B	1.5	1.06	(0.07)	0.46	(0.07)
C	15	2.55	(0.04)	2.16	(0.18)
D	150	3.46	(0.26)	3.03	(0.09)
E	1500	7.19	(1.06)	6.00	(0.32)

b) Second Experiment
Sampling Period: 0, 3, 5, 7 days.

Treatments	P Added	Days	Final Values Control X	SD	Colonized X	SD
A	0					
		3	0.018	(0.009)	0.006	(0.004)
		5	0.055	(0.012)	0.008	(0.005)
		7	0.039	(0.024)	0.022	(0.003)
			Control X	SD	Colonized X	SD
B	1.5					
		3	1.21	(0.05)	0.52	(0.05)
		5	1.32	(0.08)	0.06	(0.01)
		7	1.24	(0.19)	0.1	(0.01)

c) Third Experiment
Sampling Period: 0 and 7 days.

Treatments	P Added	Final Values Control X	SD	Colonized X	SD
A	0	0.18	(0.06)	0.85	(0.03)
Z	750	4.83	(0.88)	4.07	(1.02)
E	1500	4.76	(0.64)	5.14	(0.68)

Results were analyzed with the Dunnett's test for a two way ANOVA (Snedecor and Cochran 1977). Logarithmic transformation was applied when it was necessary.

RESULTS AND DISCUSSION

In the first experiment, mean periphyton growth was highest for treatment B, although there were no significant differences with treatment A. This result could be explained by the very high SRP concentration naturally found in the stream water, so that enrichment did not produce a noteworthy effect on final biomass after a week. With higher phosphorus addition

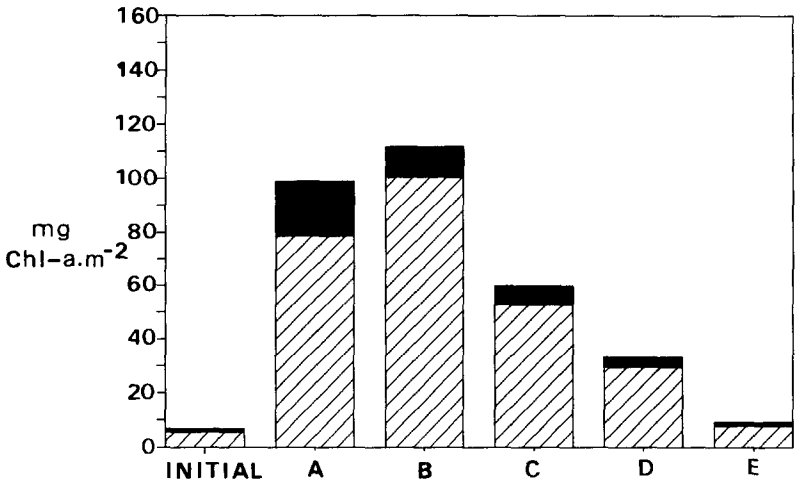

Figure 1. Mean periphyton biomass and standard error (in mg Chl-a. m^{-2}) under different phosphorus additions in days 0 and 7 in the first experiment A: No P added, B: 1.5 mg P added, C: 15 mg P added, D: 150 mg P added, E: 1500 mg P added ($p<0.01$).

(treatments C, D and E), algal growth was lower, differing significantly ($P < 0.01$) from the other groups (Fig. 1). Phosphorus absorption occurred in all treatments (Table 1). Periphyton were dominated by species of Bacillariophyceae (diatoms) in all treatments at the end of the experiment. Algal richness was similar to the initial value in treatment B, but lower in the other groups. In groups D and E, algal richness was very low (Table 2).

In the second experiment, biomass was significantly different ($P < 0.01$) between groups A and B for all sampling days (Fig. 2). Phosphorus was completely absorbed by day 3 in group A and by day 5 in group B, although increases of biomass were observed until day 5 in group A and day 7, the end of the experiment, in group B. Therefore, phosphorus influence on the growth of algae seems to last longer than the actual period of exposure to the nutrients (Table 1 and Fig. 2).

In the third experiment significant differences ($P<0.01$) in algal development among groups A, Z, and E were found. Results from Dunnett's test indicated that after seven days, periphyton biomass increased in groups A and Z, while in group E this increase was low and not significant. Phosphorus absorption was observed after seven days in treatments Z and E both in colonized and control vessels.

Table 2. Algal richness with different levels of phosphorus in the first experiment. r: rare, s: scarce, f: frequent, a: abundant, ha: high abundance.

Organisms	Initial	Groups A	B	C	D	E
BACILLARIOPHYCEAE						
Amphora veneta (Kütz)	s		f			
Achnantes flexella (Kütz) Grun	f	a	s	f	s	
Cymbella minuta Hilse ex Rabh	ha			r		
Denticula elegans Kützing			r			
G. angustatum (Kütz) Rzbh.	s	f	s			
Gomphonema constrictum Ehr.		f	r			
Gomphonema parvulum (Kütz) Kütz	r		r			
Navicula cari Ehr.	s			a		
Navicula mobiliensis Boyer		f	r			
Navicula mutica Kütz				f	f	
Navicula pupula Kütz	ha	f	r			
Navicula sp Bory	a		s			
Nitzchia palea (Kütz) W. Smith	f	a	f	ha	ha	a
Nitzchia paleaceae Grun	s		a			
Nitzchia parvula Lewis			a			
N. tryblionella (W. Smith) Grun	s					
Roicosphenia curvata (Kütz) Grun	s					
Surirella ovalis Brebison		r				
Synedra ulna (Nitz.) Ehr		r	f	f		
CHLOROPHYCEAE						
Chlorella sp Beliernick	f	f				
Cladophora sp Kützing	s					
Desmococcus vulgaris Brand	f					
Euglena sp Ehr.			f			
Oedogonium sp Link		r		r		
CYANOPHYCEAE						
Lyngbia sp Ag.	s					
Algal richness	16	10	15	8	3	1

Bothwell (1989) described three phases in the response of algae to phosphorus enrichment. The first one between 0 and 1 μg $P.L^{-1}$ is characterized by a rapid initial biomass increase which approached saturation at 0.5 μg $P.L^{-1}$; the shape of the response curve during this phase is similar to a Michaelis-Menten curve. The second one, between 2 and 28 μgP. L^{-1}, is characterized by a slow linear increase. In the third one, beyond a phosphorus concentration of 30-50 μgP.1^{-1}, biomass no longer responds to further nutrient concentration increase. According to the phosphorus concentration found, Gutierrez stream should be placed in Bothwell's phase

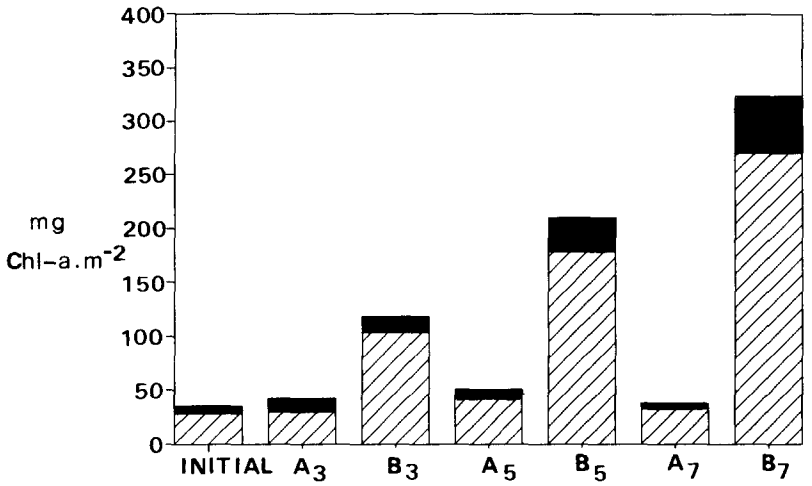

Figure 2. Results of the second experiment. Mean periphyton biomass and standard error (in mg Chl-a.m^{-2}) after 0, 3, 5, 7 days. Treatment A: without phosphorus added; Treatment B: with 1.5 mg P added.

III. The response of periphyton, evaluated as biomass increase,was different at each level of phosphorus addition. The highest biomass increase was found with an enrichment of 1,5 mg P. L^{-1}.

Periphyton growth was inhibited with an enrichment of 1500 mg P.L^{-1}, a level higher than that reported by Bothwell (1989). With an enrichment of 750 mg P.L^{-1}, periphyton response was low; so, high levels of phosphorus enrichment can reduce or inhibit algal growth.

It is possible that algae absorb and store P into the cell. As observed in the second experiment in group B, all the phosphorus was absorbed by periphyton after 5 days; although periphyton growth continued until day 7. In this sense, Payne et al.(1988) detected an increased accumulation of phosphorus in relation to high P additions.

Considering that the periphyton community of cattail stems was dominanted by diatoms in number and species, and that the period of colonization of 15 days used in this set of experiments is a short one for chlorophytes and cyanophytes, it was not surprising that the initial communities were dominanted by species of diatoms.
In general, algal richness was low in the presence of phosphorus enrichment. Only one species of diatom (Nitzchia palea) appeared to prefer high phosphorus levels.This diatom, as Germain (1981) observed, supports high degrees of pollution and shows a wide range of size

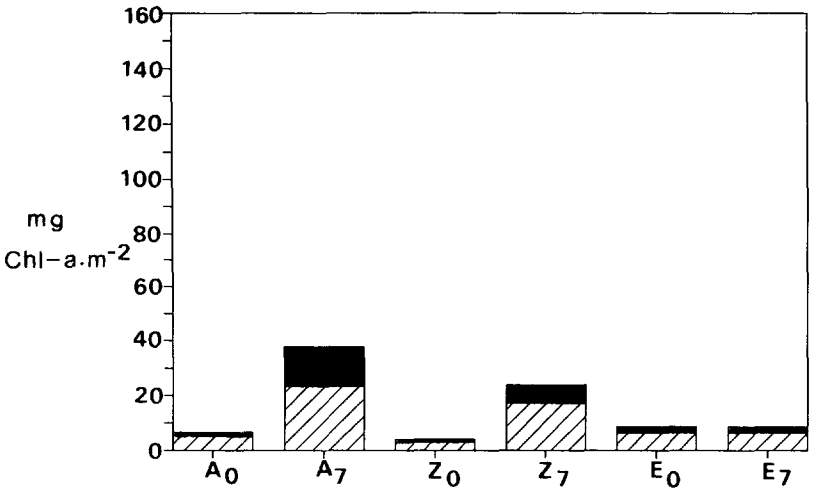

Figure 3. Mean periphyton biomass and standard error (in mg Chl-a. m^2) under different phosphorus additions in days 0 and 7 in the third experiment. Treatment A: without phosphorus added; Treatment Z: with 750 mg P added; Treatment E: with 1500 mg P added (0:initial biomass; 7: final biomass.($p<0.01$).

variation. Phosphorus defficiency also reduces algal richness as observed in group A after 7 days in the first experiment.

Continuous illumination will produce photoinhibition of respiration which can be reduced about 40 % (Graham and Turner 1987). Consequently, algae growth should be higher than those obtained in these experiments.

However, this study shows a broad physiological adaptation of algae to phosphorus concentrations. Periphyton absorbs phosphorus even at very high SRP concentrations; on the other hand, this study suggests that growth should be inhibited at the phosphorus concentrations found in urban effluents.

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