AVAILABILITY OF PHOSPHORUS-32, ADSORBED ON CLAY PARTICLES, TO A GREEN ALGA

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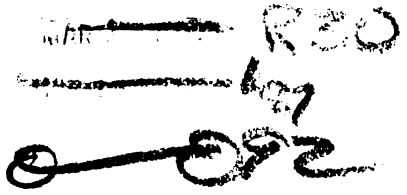

ABSTRACT

AVAILABILITY OF PHOSPHORUS-32, ADSORBED ON CLAY PARTICLES, TO A GREEN ALGA

By

Louis A. Helfrich

The exchange of radiophosphorus between clay particles, culture medium and phosphate-limited Pandorina morum cells was examined in order to determine the availability of phosphorus adsorbed on clay particles to algal cells. The cultures were maintained in three light regimes in an attempt to equate the uptake and release of ^{32}P with the pH and dissolved oxygen concentrations associated with the fluctuating light conditions.

Of the total amount of adsorbed ^{32}P present in all the cultures a mean uptake of approximately 30% was found in the cells after the second day. The greatest uptake of the tracer by the algal cells occurred in the cultures which received constant light and exhibited the highest dissolved oxygen levels as well as the highest pH values. the greatest loss of adsorbed ^{32}P by the clay was found in those cultures which received no light and

exhibited the lowest dissolved oxygen levels and the lowest pH values.

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Louis A. Helfrich

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INTRODUCTION

Recently, a great deal of concern has been expressed about the rapid eutrophication of many bodies of water manifested by nuisance growths of aquatic vegetation. Although many nutrients can potentially contribute to such undesirable productivity, phosphorus has been established as the nutrient which is most often limiting to such growth (Mackenthun, 1968).

Phosphorus found in surface waters may originate from a variety of sources that include; sewage effluents, synthetic detergents, industrial wastes, and drainage from agricultural lands. An initial concern in reducing the phosphate problem in lakes and streams is to identify the magnitude of phosphorus sources and the relative availability of phosphorus as a plant nutrient from the various sources. Much emphasis has been placed on the removal of phosphorus from wastewaters and detergents to effectively control the phosphorus concentrations and reduce environmental degradation. While implementation of this approach and technology can substantially reduce phosphorus levels

in some natural waters it may not reduce plant production if the contribution of phosphorus from land drainage is sufficiently available for plant growth.

This research was conducted to determine if phosphate adsorbed on clay particles, as representative of typical drainage water sediments, is available to phosphate-limited Pandorina morum and to examine some of the parameters which influence the availability of this nutrient. More definite information about the quantities of adsorbed phosphorus available for plant growth and the mechanisms which control this availability may ultimately lead to effective control of eutrophication.

In the terrestrial watershed virtually all of the soluble phosphorus is readily fixed by silts, clays and clay minerals and is often considered unavailable for plant use (Murphy, 1939; Low and Black, 1948; Hsu, 1960). Despite fixation on soils, phosphorus is often conveyed in appreciable quantities to natural waters adsorbed on suspended soil particles, particularly clay, as a result of land drainage and erosion. Johnson and Moldenhauer (1970) suggest that enormous quantities of suspended sediments, often carrying water pollutants such as plant nutrients, are delivered to streams and lakes as a result of erosion. Other investigators have concluded that land drainage is a significant source of phosphates in streams which drain agricultural lands and that rural runoff may

be a major factor in stream phosphate pollution (Engelbracht and Morgan, 1961; Weidner, et al., 1969). Kohnke and Bertrand (1959) indicate that typical surface runoff is high in clay particles and adsorbed phosphates. Suspended sediment may play a major role in controlling the dissolved phosphorus concentrations in natural waters by providing sorptive sites. Keup (1968) concluded that significant quantities of phosphorus may be transported in flowing waters as bed-loads or with floating materials.

Although phosphorus definitely reaches aquatic environments via runoff the availability of adsorbed forms of this nutrient to aquatic plants remains obscure. Several investigators reason that the amount of phosphate in sorptive chemical exchanges between the sediments and water is large enough to be significant in biological processes (Pomeroy, et al., 1965; Harter, 1968). Golterman, et al. (1969) reported excellent growth of Scenedesmus obliquus was obtained with mud as a sole phosphorus source. This paper establishes that phosphorus adsorbed to clay particles is available to Pandorina morum cells.

METHODS AND PROCEDURES

Pure cultures of Pandorina morum (18) obtained from the culture collection of Indiana University were used to examine the availability of radioactive phosphorus (^{32}P) adsorbed on clay particles.

Culture Conditions

All glassware used in experiments was washed in potassium dichromate-sulphuric acid solution and successively rinsed in double-distilled water. In order to prevent contamination from dust and to permit gas exchange, the flasks were stoppered with polyurethane foam plugs. Both the glassware and the culture medium were autoclaved for 30 minutes at 15 psi, and all transfers, innoculations and samples were taken with sterile serological pipetts in order to retard bacterial growth.

Axenic cultures of stock P. morum were grown in 150 ml erlenmyer flasks placed within culture chambers designed for the regulation of photoperiod and temperature (Figure 1). The conditions in the flasks were: solution volume, 100 ml; temperature, 27±2 C; light intensity

Figure 1. Photograph of the culture chamber designed for light and temperature control.

provided by gro-lux fluorescent lights, 120 ft^{-}c; agitation daily by hand, and initially equivalent nutrient concentrations. The culture medium, Chu-10 (Chu, 1942), was modified to double strength in all major nutrients to ensure adequate growth (Table 1). A week prior to the tests these cells were transferred after centrifugation to a series of flasks containing the modified Chu-10 medium with all nutrients except phosphorus, insuring that the cells would become limited in phosphate during subsequent growth.

In an attempt to examine the effects of light on the uptake of adsorbed ^{32}P by the algal cells a series of nine flasks was established according to a factorial design (3 cultures X 3 light regimes). Thus one-third of the cultures each received 24 hours of light per day, 12 hours of light per day, and the final third was maintained without light. Control cultures in each of the light regimes were used to monitor changes in pH and dissolved oxygen, while other control flasks without algal cells were used to demonstrate changes in the phosphorus equilibrium between the medium and the clay particles.

Measurement of Growth, pH and DO

With differing light regimes, corresponding changes in algal growth, pH, and dissolved oxygen concentrations of the cultures were examined to gain some insight on the

TABLE 1.--Composition of Modified Chu-10 Medium (Chu, 1942).

Nutrients	Concentration
Major	mg/l
$Ca(NO_3)_2 \cdot 4H_2O$	40.0
$MgSO_4 \cdot 7H_2O$	80.0
K_2HPO_4	10.0
$NaHCO_3$	40.0
Trace	
NaETDA	3.00
$FeCl_3 \cdot 6H_2O$	0.08
$MnCl_2 \cdot 4H_2O$	0.06
$ZnCl_2$	0.01
$CoCl_2 \cdot 6H_2O$	0.002
$NaMoO_4 \cdot 2H_2O$	0.01
Vitamins*	
B_{12}	0.03
Biotin	0.0003
Thiamin . HCl	0.00003

*Modified by Dr. Brian Moss (personal communication).

release and uptake of ^{32}P. Algal growth (standing crop) was determined gravimetrically to the nearest milligram. The pH changes were monitored by meter to the nearest tenth of a unit.

The sodium azide modification of the Winkler method was used for dissolved oxygen determination. Fromm (1958) developed a semi-micro procedure for measuring the dissolved oxygen concentration. This method is rapid, accurate, and allows for determinations in a small volume of water. The $MnSO_4$ solution, alkaline KI solution and phosphoric acid in the usual concentrations were conveniently stored in rubber-stoppered serum bottles. Phosphoric acid was substituted for sulfuric acid in these micro-determinations as sulfuric acid may cause the liberation of iodine from the alkaline KI solution (Fox and Wingfield, 1938).

Clay Preparation

Kaolinite, a well known descriptive type clay mineral whose role in phosphate fixation in soils has been established, was used to represent typical suspended and settleable sediments in aquatic systems. A 5% suspension of kaolinite was homogenized in a blender and this suspension was slowly introduced into an ion exchange column containing a medium porosity Amberlite IR-120 resin. This exchange replaces Ca ions with H ions. The addition of 1N NaOH to the eluant maintained the pH above 8.5 as

well as replacing the H ions with Na ions. In order to obtain a relatively uniform particle size the Na-clay suspension was vigorously shaken and allowed to settle. Stokes's Law (Beaver, 1956) describes this settling as a function of the relationship between the radius of a particle and its rate of fall in a liquid. Reionization of the clay suspension replaced the Na ions with H ions, and by spontaneous decomposition of the clay surface the H-clay is transformed into an acidic Al-clay. Clay particles prepared in this manner readily adsorb phosphorus.

The tracer, carrier-free ^{32}P as orthophosphate in 0.02 M HCl, was added to the clay suspension which was continually stirred for three days to insure that a large percentage of the tracer was adsorbed to the clay. Experiments were initiated by adding 5 ml of the radioactive clay, calculated to give about 8,000 cpm/ml in the cultures.

Radiological Techniques

Radiophosphorus, ^{32}P, was chosen as the tracer because it provides accurate measurements of the phosphorus concentrations in each of the three major categories; culture medium, clay particles, and algal cells. This isotope emits a single high energy beta particle (1.712 mev), has excellent tracer qualities, and a satisfactory half-life (14.3 days).

Radioactivity was measured with an internal gas flow, end-window gieger detector equipped with a low background anti-concidence unit. To insure consistent counting geometry all samples were mounted in polished steel planchets providing a total area of 2.54 cm^2. In order to facilitate uniform distribution of the sample in the planchets two drops of normal hexane were added during the drying process. Drying of the samples was accomplished using a hot plate at 105 C. The counting efficiency, determined daily, averaged about 38.25% for a simulated ^{32}P standard of natural uranium. Counting times were determined to be significant at the 95% level (Overman and Clark, 1960). Background, sample geometry and back-scattering all remained relatively constant. Preliminary experiments demonstrated that self adsorption, in such small samples along with a high energy tracer, was negligible. All measurements were corrected for counter dead time and radioactive decay (Appendix A).

Sampling Procedure

The experimental flasks, incubated under the same conditions as the control cultures, were removed from the chambers at the end of the dark period every day and thoroughly swirled. A 10 ml sample was taken from each culture with a pipette. From each of these samples a one milliliter aliquot was dried by evaporation and counted to determine the activity in the whole sample. The remaining

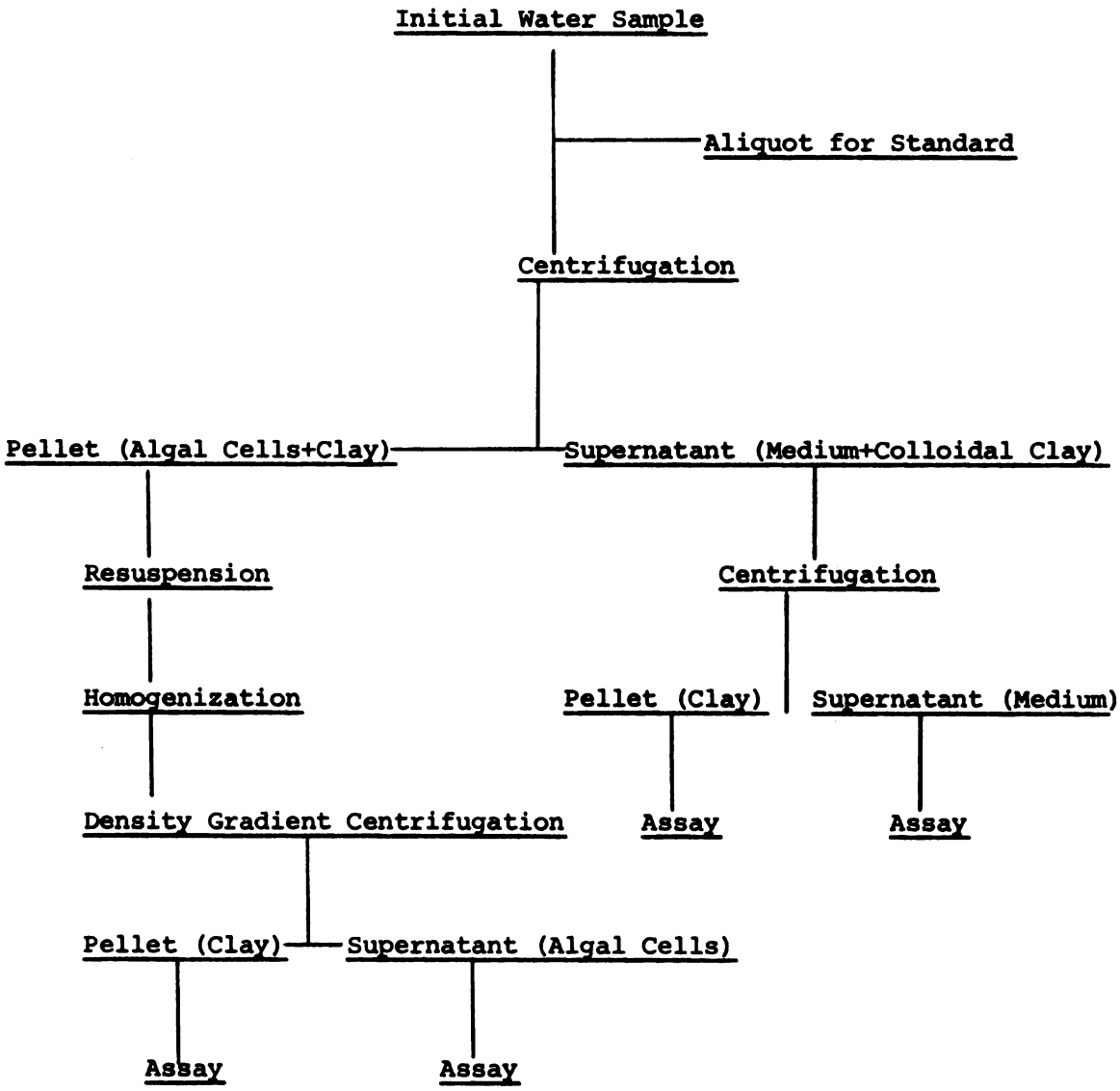
9 ml of the sample was fractionated for further isotope analysis. From each of the control cultures an 8 ml water sample was drawn into a syringe and used to determine dissolved oxygen concentrations. The flask was then swirled and the pH of the culture determined.

Sample Fractionation

Centrifugation and density gradient separation procedures were used to fractionate the water sample into three major categories: culture medium, clay particles and algal cells. These methods allow for the concentration and separation of the sample into homogeneous fractions in order to determine the quantative distribution of ^{32}P in each category.

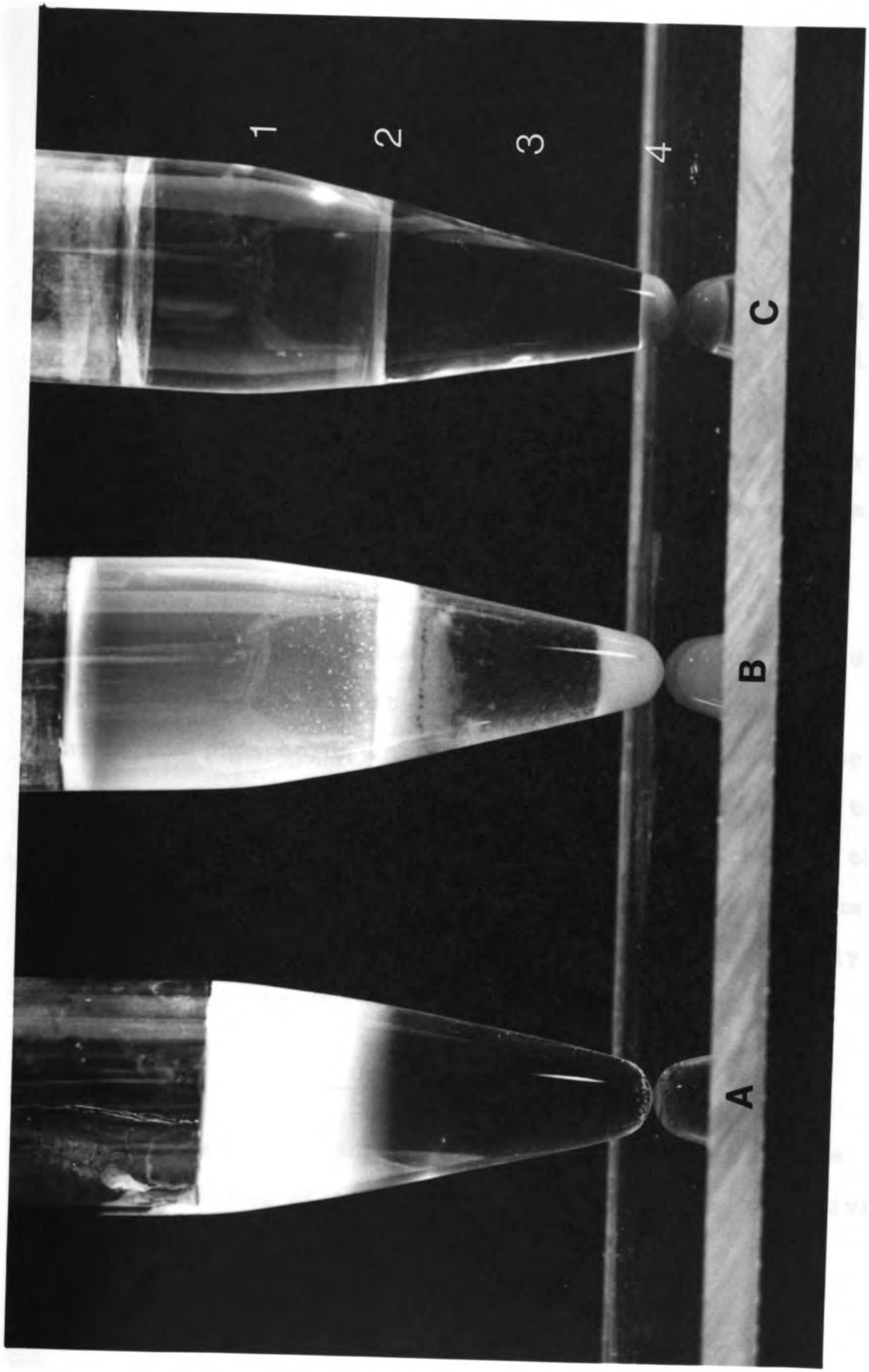
Lamers (1966) developed a scheme for natural water fractionation which was modified for use in this study (Figure 2). The samples were initially separated into two fractions by centrifugation at 2500 rpm for 20 minutes. The supernatant culture medium and any colloidal clay was removed by cautious aspiration, separated by centrifugation at 9000 rpm for 20 minutes and prepared for radionuclide analysis. The remaining fraction of algal cells and clay particles was resuspended in isotope free medium and homogenized. This suspension was slowly introduced over 2-bromoethanol (density 1.77, viscosity 4.5 cp) and centrifuged at 2500 rpm for 20 minutes. Bromoethanol is a water soluble alcohol which forms a density gradient

Figure 2. A modified flow diagram for water fractionation (Lamers, 1966).


Initial Water Sample
Aliquot for Standard
Centrifugation
Pellet (Algal Cells+Clay)
Supernatant (Medium+Colloidal Clay)
Resuspension
Centrifugation
Homogenization
Pellet (Clay)
Supernatant (Medium)
Density Gradient Centrifugation
Assay
Assay
Pellet (Clay)
Supernatant (Algal Cells)
Assay
Assay

through which the denser clay particles pass, but the gradient has a higher density than the algal cells, effectively fractionating the sample (Figure 3). Each fraction of the sample was removed by cautious aspiration and dried for analysis.

Figure 3. Photograph of sample fractionation in a bromoethanol density gradient showing medium zone (A), algal cells (B), bromoethanol (C), and clay zone (D).


1
2
3
4
A
B
C

RESULTS

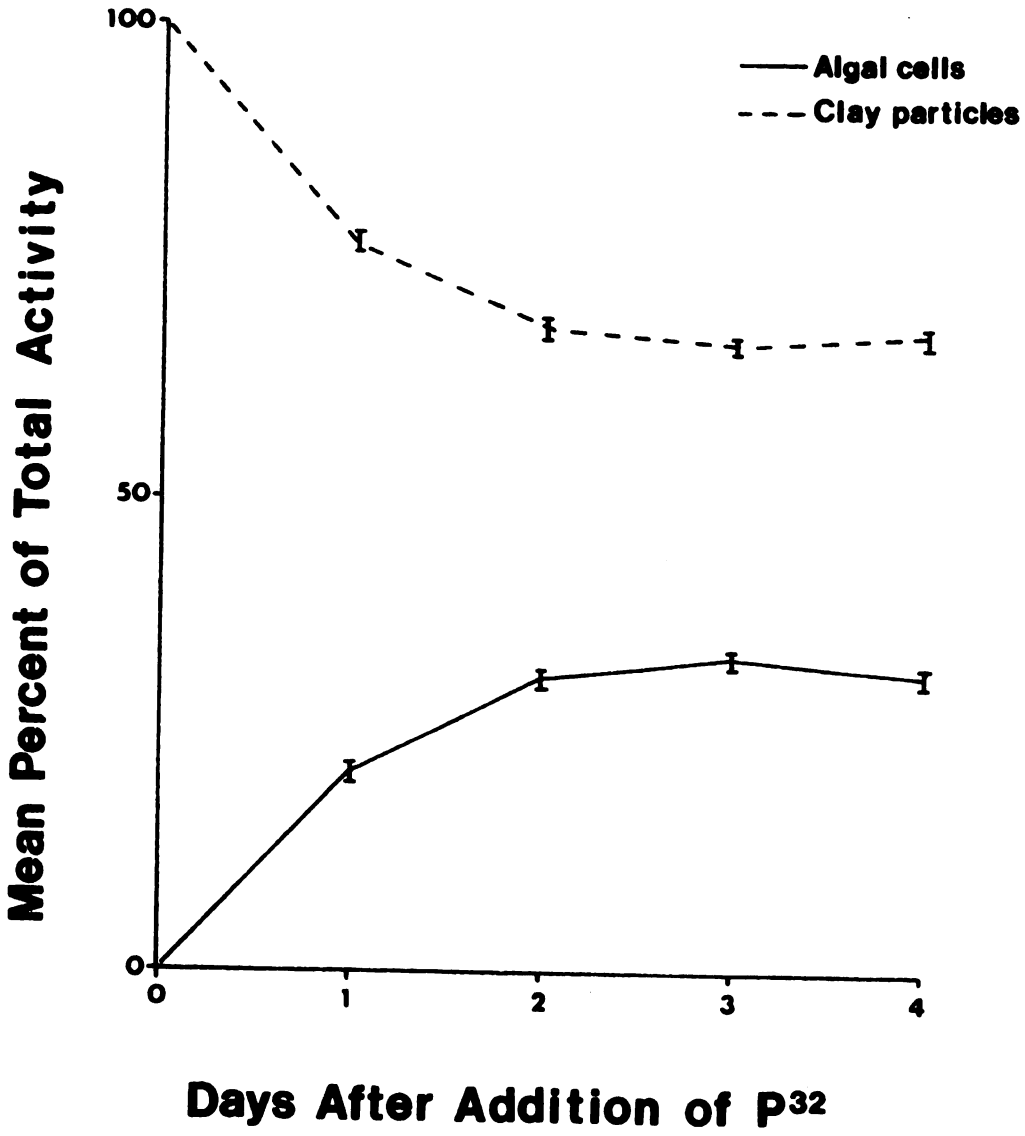
Radioactive phosphorus, adsorbed to clay particles, is available to phosphate-limited P. morum cells. Initially all of the phosphorus introduced to the cultures was adsorbed on clay particles. Thus, any phosphorus tracer evidenced in the algal cells must have been derived from that adsorbed on the clay.

The mean percentage of the total amount of ^{32}P present in the algal cells and clay particles for each day is shown in Figure 4. It appears that an equilibrium between the phosphorus in the algal cells, aqueous phase and the clay sediments was established, without regard to light regime, after the second day. The percentage of the tracer in the algal cells increased rapidly to a maximum of approximately 30% in two days and remained relatively constant until the end of the experiment.

^{32}P Uptake by Cells and Loss From Clay

The activity sorbed by algal cells in all three light regimes was highly correlated with a loss of activity

Figure 4. Mean percent of total activity for P. morum and clay as a function of time, showing the 95% confidence limits.


100
50
0
0
1
2
3
4
Algal cells
Clay particles
Mean Percent of Total Activity
Days After Addition of P32

from the clay particles. As was expected, a negative correlation was shown describing the compensatory phenomenon of a loss of ^{32}P from the clay particles with a subsequent gain of the tracer by the algal cells. Those cultures maintained under constant light (complete darkness and constant light) showed the highest correlations with r values of -0.90 and -0.82 respectively. These values were significantly different from 0 at the 0.01 level. The uptake of ^{32}P by the cells in the 12-hour light regime also was correlated with the loss of activity from the clay sediments having a correlation coefficient of -0.67, which was significant at the 0.05 level. The degree to which the loss of the isotope by the clay is correlated with a gain in activity by the cells is illustrated for each light regime (Figures 5, 6, 7). The shape of the ellipse is a function of the correlation between the two variables (loss by clay versus gain by algal cells), and the area of the ellipse is a function of the confidence coefficient (Sokal and Rohlf, 1969).

Effects of Light and Time on ^{32}P Uptake

An attempt was made to follow the fate of adsorbed radiophosphorus in order to distinguish any differences in the concentration of the tracer in the cells as a function of light or time. The mean activity found in the algal cells for each of the three light regimes during the four

Figure 5. Mean activity for P. morum versus activity in the clay over four days with continuous light.

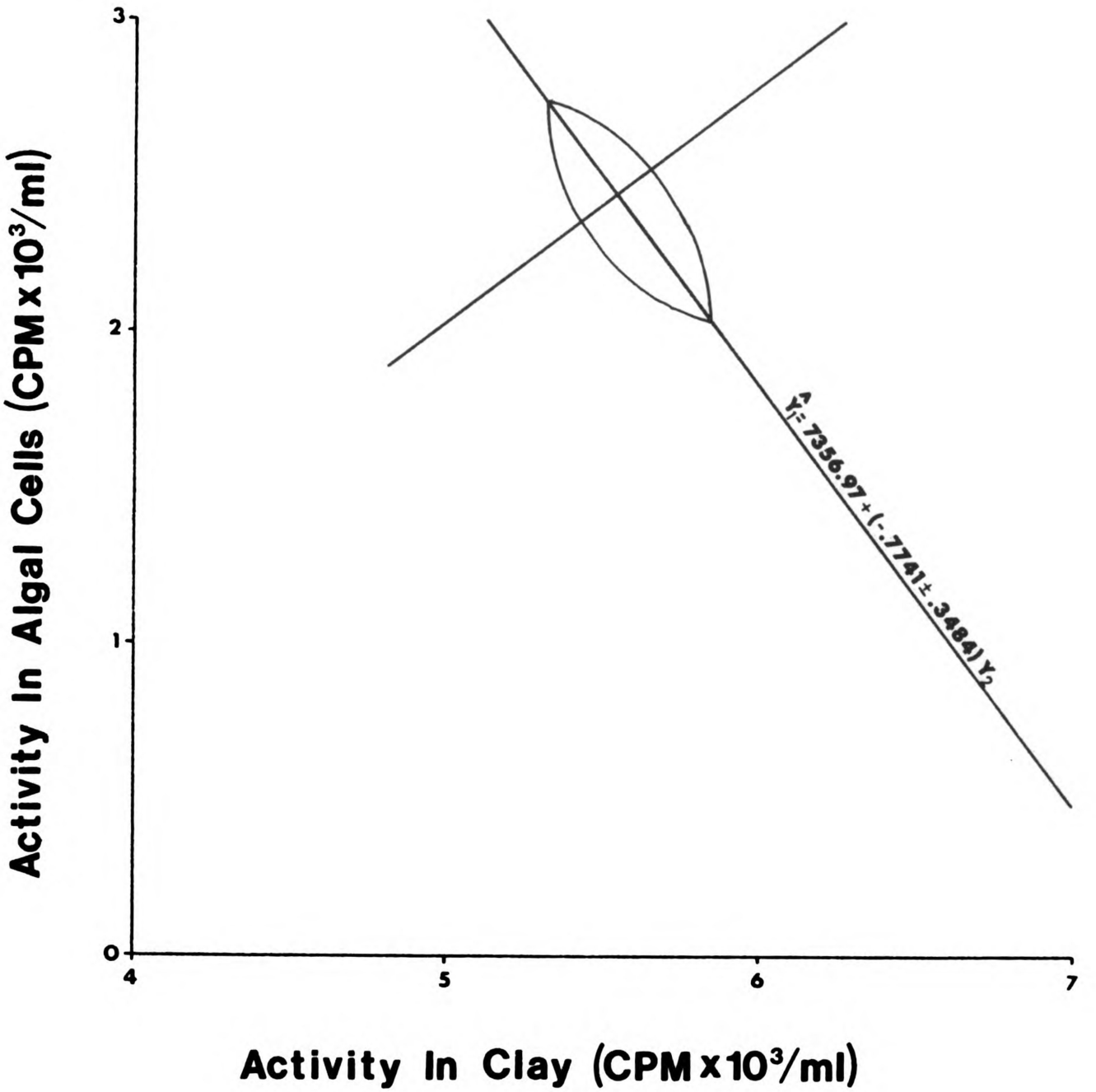

Activity In Algal Cells (CPM x 10³/ml)
3
2
1
0
4
5
6
7
Activity In Clay (CPM x 10³/ml)
$\hat{Y}_1 = 7356.97 + (-.7741 \pm .3484) Y_2$

Figure 6. Mean activity for P. morum versus activity in the clay over four days with a 12-hour light regime.

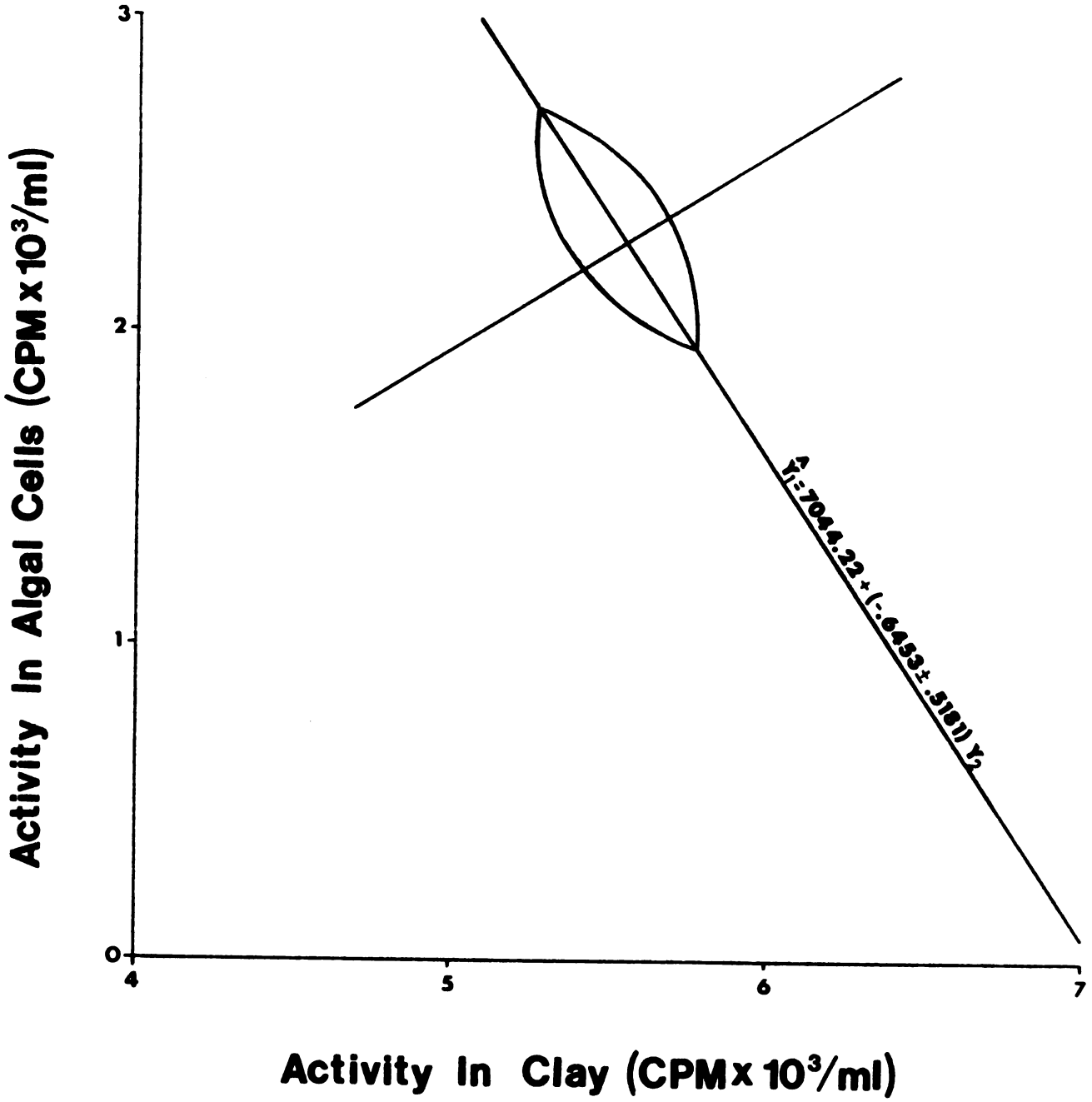
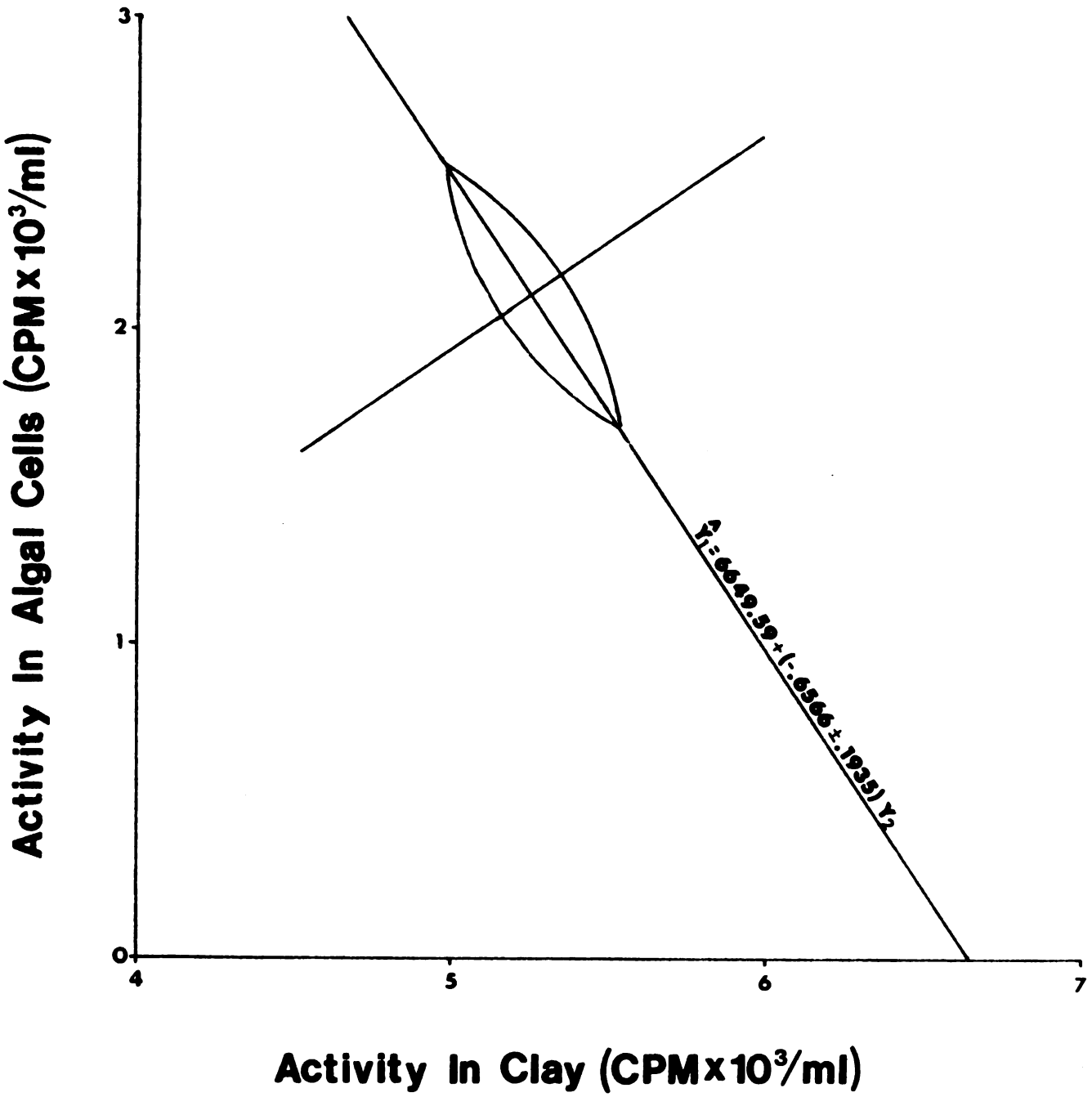

Activity In Algal Cells (CPM x 10³/ml)
3
2
1
0
4
5
6
7
Activity In Clay (CPM x 10³/ml)
Ŷi=7044.22+(-.6453±.1811) Xi

Figure 7. Mean activity for P. morum versus activity in the clay over four days with no light.


Activity In Algal Cells (CPM x 10³/ml)
3
2
1
0
4
5
6
7
Activity In Clay (CPM x 10³/ml)
Ŷ₁ = 6649.59 + (-.6566 ± .1935) Y₂

days was determined (Figure 8). The standard error and coefficient of variation of the cellular activity for each photoperiod is shown in Appendix B. An almost linear increase in cell activity was shown in all three light regimes as the experiment proceeded. Those cultures which received constant light exhibited the greatest uptake of the tracer, reaching a maximum concentration of approximately 2900 cpm on the third day.

In order to further investigate the effects of light on the activity sorbed by algal cells a one-way analysis of variance was conducted for each of the four days (Appendix C). Ducan's multiple-range test was used for comparing treatment means. The activity in the cells was significantly different ($P<.05$) for all light conditions on each day except the third when no significant difference in algal activity was determined between the cultures in the 12-hour light regime and those which received no light.

Effects of Light and Time on ^{32}P Release

The mean activity of the clay particles for each light regime during the four days is shown in Figure 9. The standard error and coefficient of variation of the activity in the clay for each light regime during the four days is given in Appendix D. A rapid decrease in the concentration of adsorbed ^{32}P was shown during the

Figure 8. Mean activity for P. morum in each light regime as a function of days after introduction of ^{32}P adsorbed to clay particles.

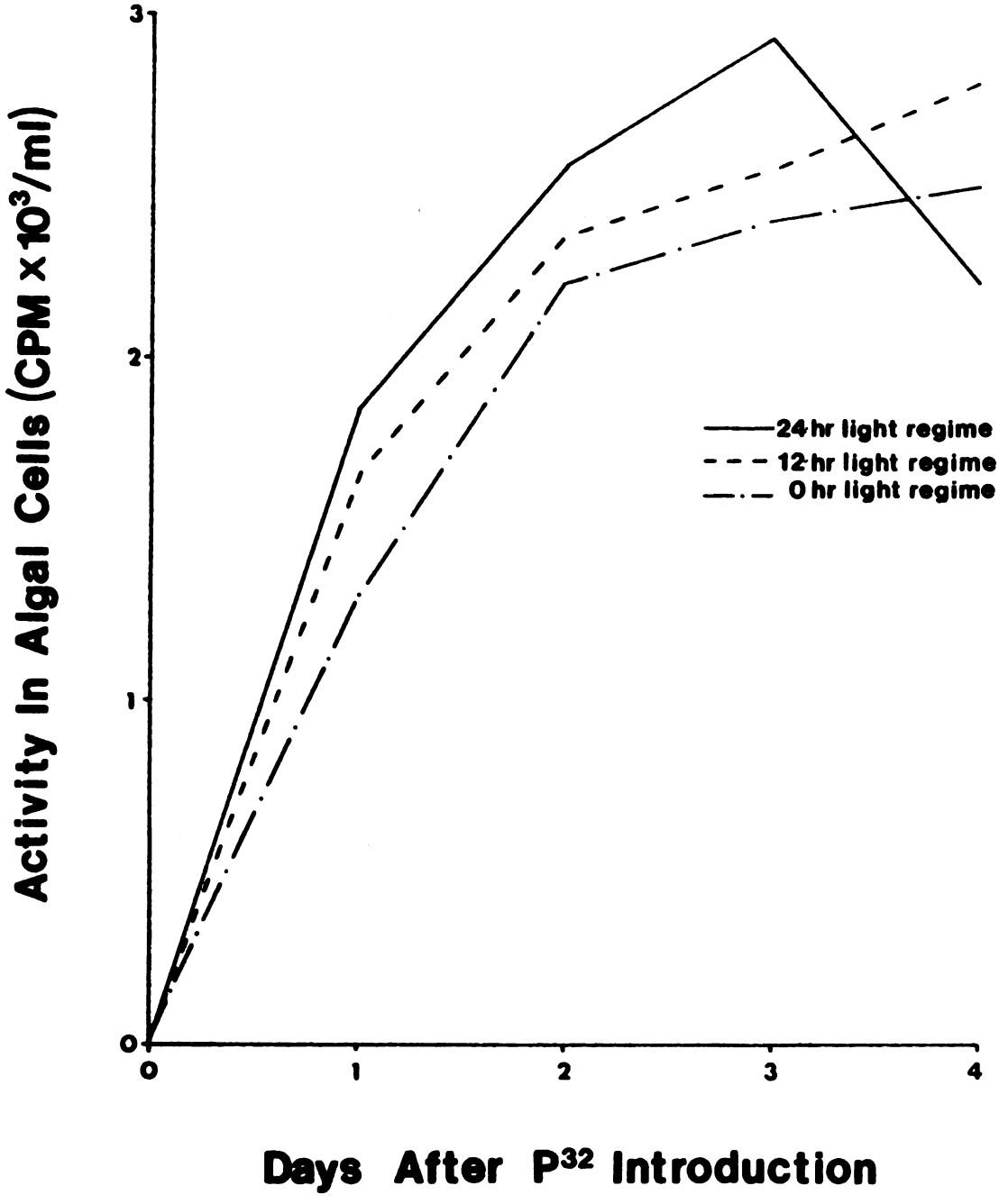
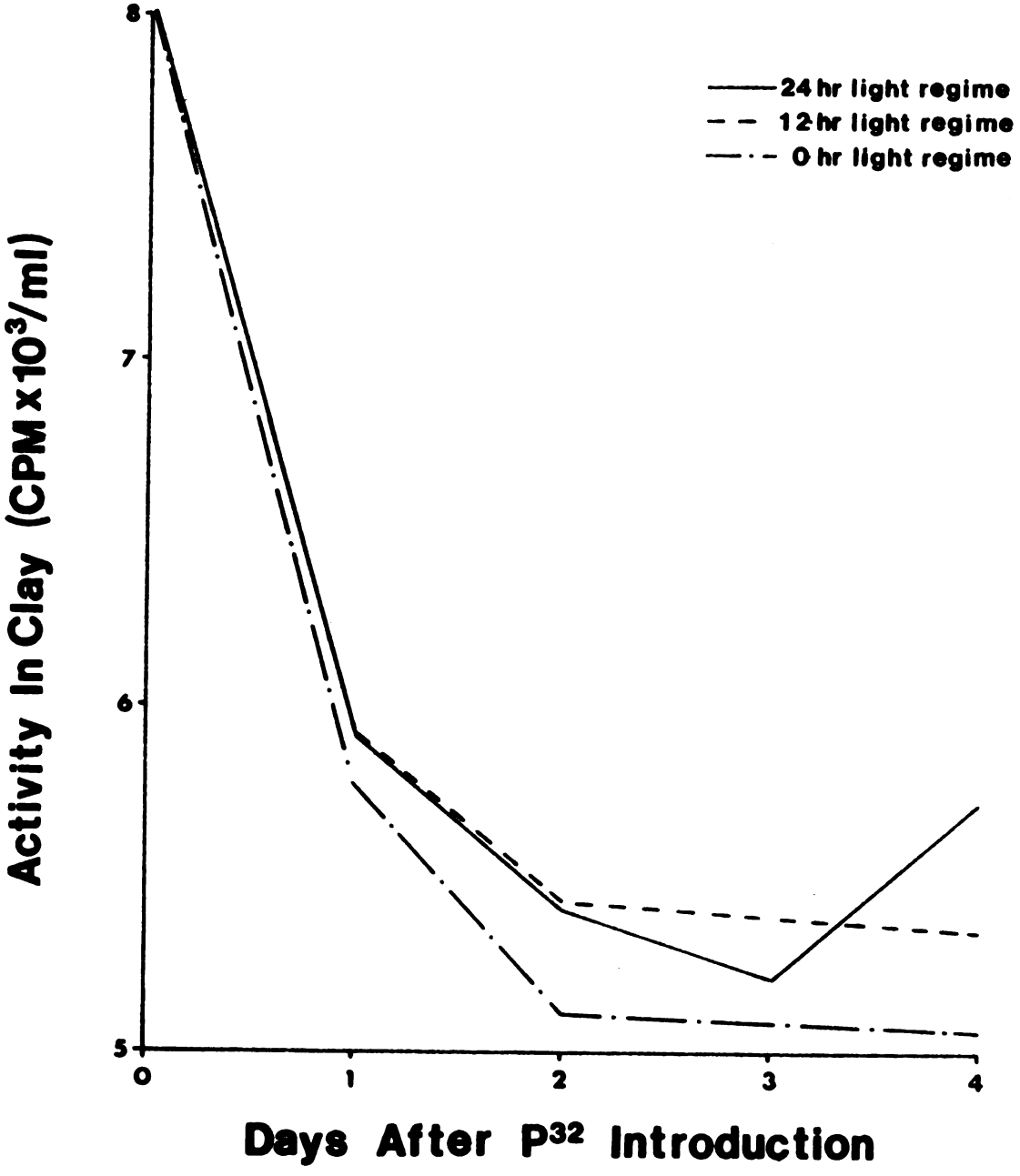

Activity In Algal Cells (CPM x 10³/ml)
3
2
1
0
0
1
2
3
4
24 hr light regime
12 hr light regime
0 hr light regime
Days After P³² Introduction

Figure 9. Mean activity in clay sediments for each light regime as a function of days after introduction of ^{32}P adsorbed to clay particles.


24 hr light regime
12 hr light regime
0 hr light regime
Activity In Clay (CPM x 10³/ml)
8
7
6
5
0
1
2
3
4
Days After P32 Introduction

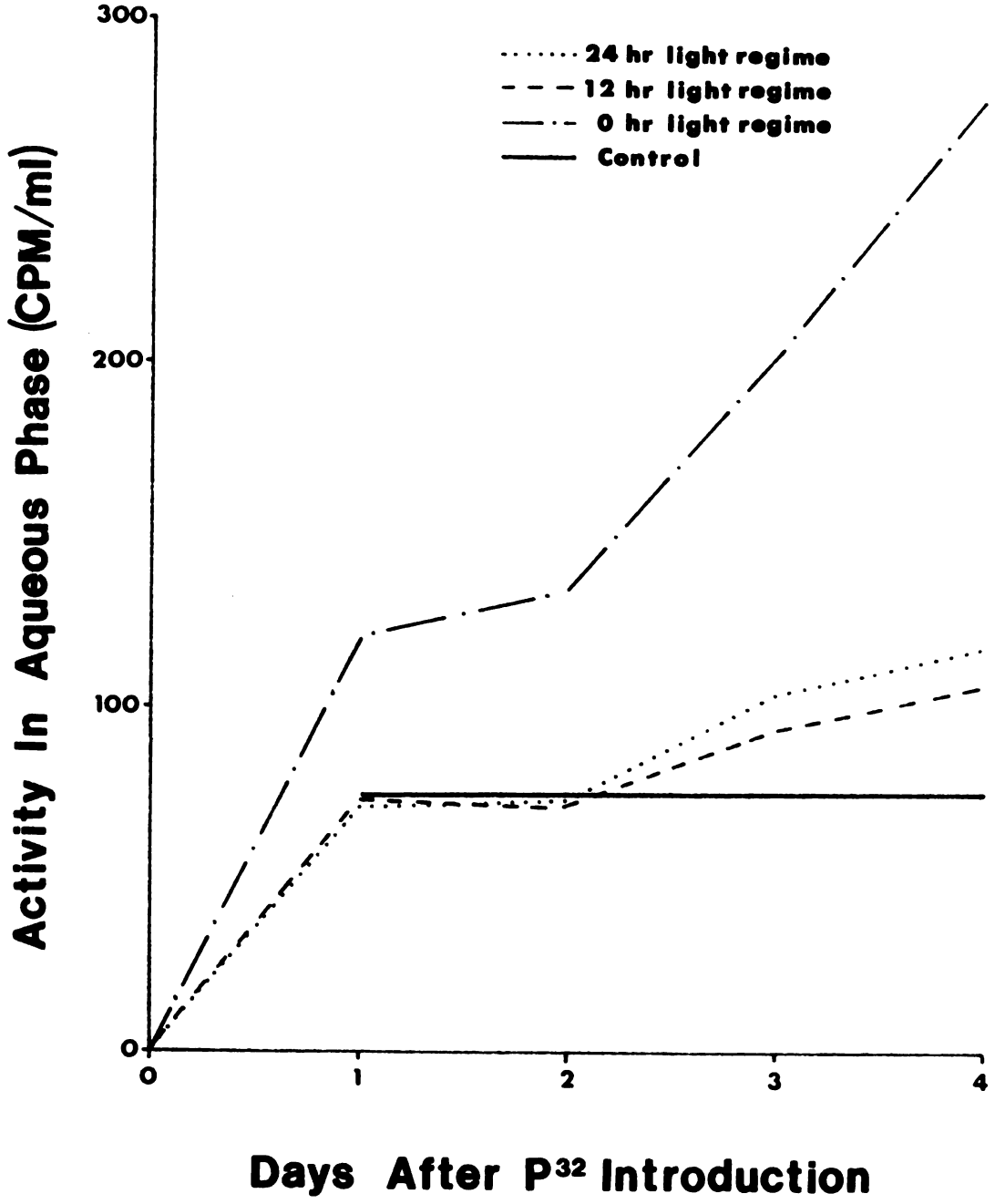
first two days. The greatest loss of phosphorus from clay sediments occurred in the cultures kept in constant darkness. A gain in activity of the clay particles on the fourth day in the 24-hour light regime occurred simultaneously with a loss of ^{32}P by the algal cells in the same light conditions.

The effects of light and time on the release of ^{32}P from the clay particles were considered simultaneously by a two-way analysis of variance (Appendix E) and the treatment means were compared with Ducan's multiple range test. A significant difference ($P<.001$) in the release of the tracer by the clay was shown among light regimes and days.

Activity in Aqueous Phase

With the introduction of relatively high concentrations of adsorbed ^{32}P, the amount of tracer in the aqueous phase increased (Figure 10). The standard error and coefficient of variation for the activity in the water is given in Appendix F. The increase in the levels of the isotope in the culture medium occurred in all three light regimes and continued for the four days. The greatest amount of activity was released to the aqueous phase by the clay particles in the cultures maintained without light. No significant difference in the amount of activity found in the aqueous phase occurred between the cultures receiving 24 hours and 12 hours of light. In

Figure 10. Mean activity in aqueous phase for each light regime as a function of days after introduction of ^{32}P adsorbed to clay particles.


24 hr light regime
12 hr light regime
0 hr light regime
Control
Activity In Aqueous Phase (CPM/ml)
300
200
100
0
0
1
2
3
4
Days After P32 Introduction

comparing the average concentrations of ^{32}P found in the culture medium with the activity assayed in the cells, it is apparent that P. morum can concentrate radiophosphorus at least 40 times that amount found in the water.

Three control flasks, without algal cells, were maintained in each light regime in order to monitor the physical equilibrium of the tracer between clay particles and the aqueous phase. The mean activity in the medium after the first day was 75 cpm/ml in all light regimes, and this value remained relatively constant throughout the experiment.

Dry and Organic Weight of Algal Cells

The dry weight of the algal cells in all light regimes remained relatively constant during the four experimental days. The mean dry and organic weight of P. morum in each light regime for the four days with standard error and coefficient of variation is shown in Table 2. No significant growth was evident in any of the light regimes during the four days; this was probably a function of the large algal samples necessary for analysis. The amount of organic matter was distributed in almost direct proportion to the dry weight. The organic matter was highly correlated with the dry weight (Appendix G) as would be expected.

TABLE 2.--Mean Dry and Organic Weight of P. morum in Each Light Regime for Four Days With Standard Error and Coefficient of Variation.

Day	Photoperiod (hrs)	Dry Weight±SE (ug)	$\frac{S(100\%)}{\bar{x}}$
I	24	228 ± 16.62	12.62
	12	210 ± 16.84	13.86
	0	245 ± 12.68	8.95
II	24	219 ± 15.84	12.54
	12	248 ± 20.23	14.13
	0	240 ± 11.67	8.40
III	24	181 ± 5.69	5.44
	12	213 ± 17.32	14.06
	0	237 ± 14.99	10.94
IV	24	185 ± 4.84	4.54
	12	219 ± 17.44	13.78
	0	183 ± 12.73	12.07

Day	Photoperiod (hrs)	Organic Weight±SE (ug)	$\frac{S(100\%)}{\bar{x}}$
I	24	204 ± 16.80	14.26
	12	184 ± 15.57	14.65
	0	226 ± 12.01	9.20
II	24	201 ± 15.39	13.26
	12	170 ± 20.42	20.75
	0	217 ± 9.60	7.64
III	24	158 ± 5.24	5.73
	12	189 ± 16.07	14.65
	0	181 ± 33.85	32.44
IV	24	161 ± 7.64	8.21
	12	204 ± 19.55	16.62
	0	161 ± 13.92	15.00

Effects of pH on ^{32}P Movement

The pH values for all three light regimes increased during the four experimental days (Figure 11). Initially all the cultures were slightly acidic caused by the introduction of the acidic clay particles. The greatest increase in pH was shown by those cultures in the 24-hour light regime. The cultures maintained in the other light regimes showed a slight increase in pH during the four days. The pH values were significantly correlated ($P<.01$) with the uptake of the tracer by the algal cells for all light regimes during the experiment. When the uptake of ^{32}P was maximum the pH in all light regimes ranged between 7.3 and 7.9.

Effects of DO on ^{32}P Movement

The concentration of dissolved oxygen in the algal cultures increased in all light regimes during this experiment (Figure 12). As was expected, the highest levels of dissolved oxygen were found in the cultures which received constant light, reaching a maximum concentration of 10.2 mg/liter on the fourth day. The oxygen levels found in the cultures maintained in the dark are lower but seem to increase proportionally with the cultures in the 12-hour light regime. The greatest release of ^{32}P occurred in the dark cultures which also evidenced the lowest oxygen concentrations.

Figure 11. Mean pH values in control cultures for each light regime during the four days.

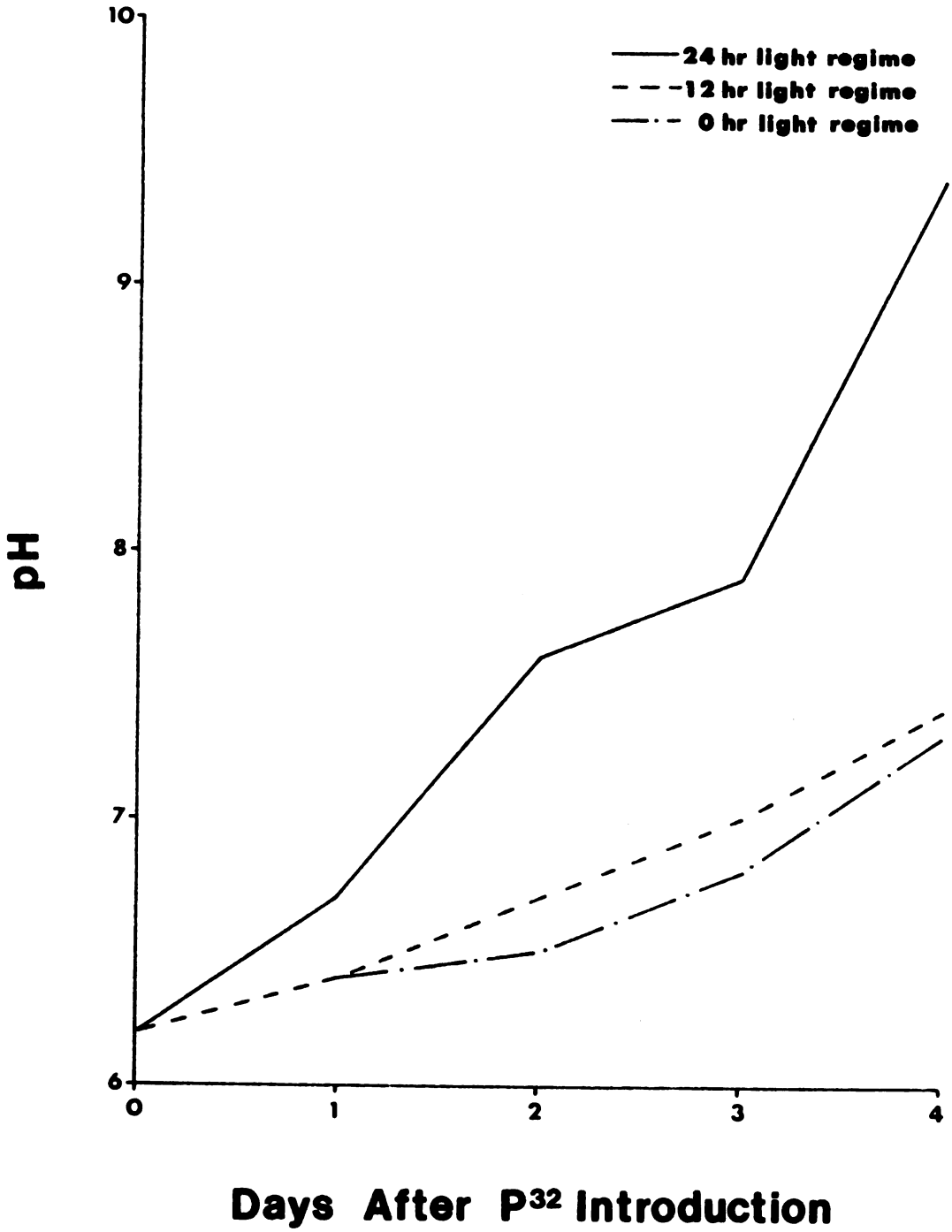
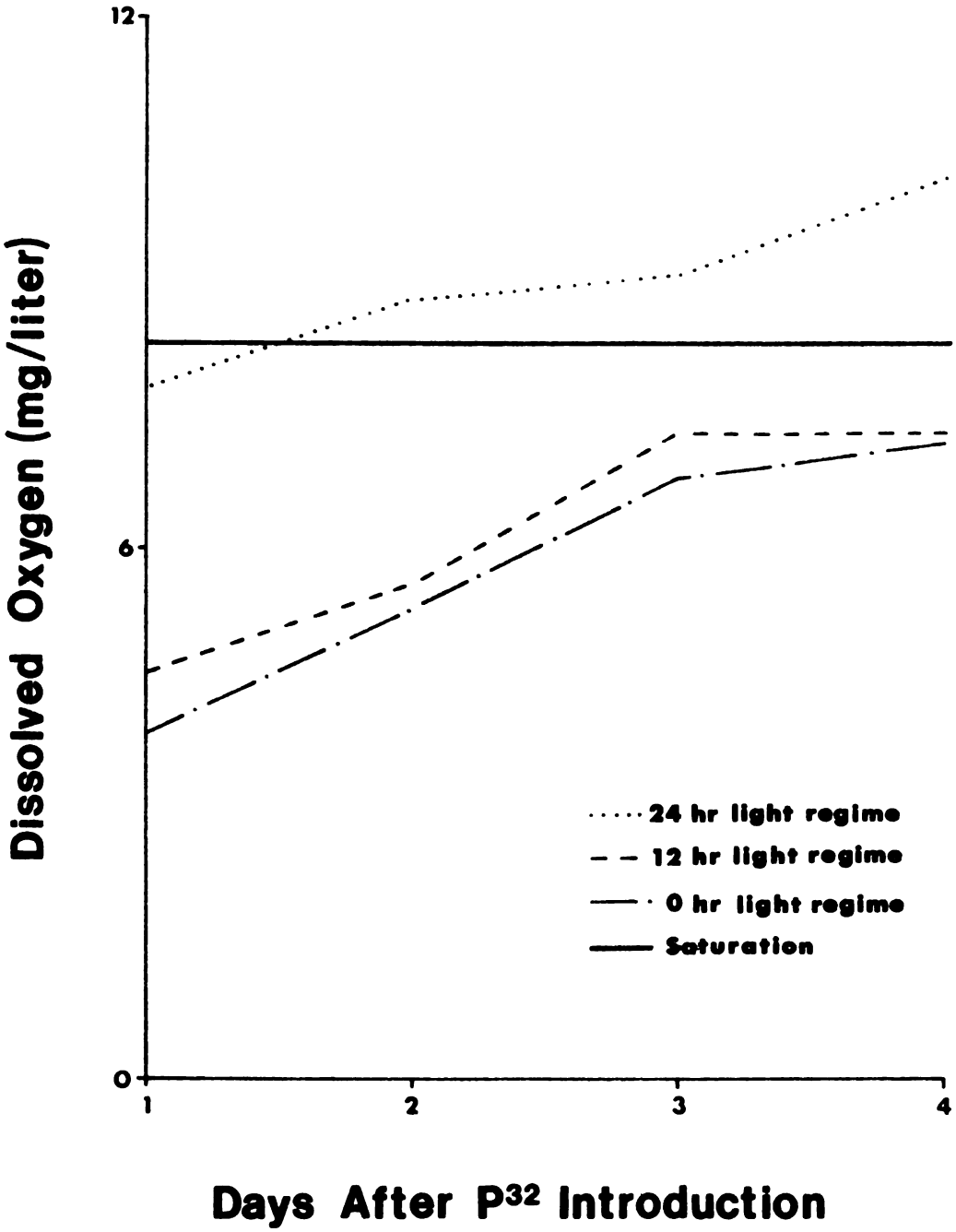

24 hr light regime
12 hr light regime
0 hr light regime
pH
10
9
8
7
6
0
1
2
3
4
Days After P32 Introduction

Figure 12. Mean dissolved oxygen concentration in the control cultures for each light regime during the four days.


12
6
0
1
2
3
4
Dissolved Oxygen (mg/liter)
24 hr light regime
12 hr light regime
0 hr light regime
Saturation
Days After P32 Introduction

DISCUSSION

The results presented in this paper serve to emphasize the potential role of clay particles in supplying phosphorus necessary for algal nutrition. That phosphate-limited P. morum can obtain this nutrient adsorbed to clay particles has been demonstrated. Originally the only source of activity in the cultures was as ^{32}P adsorbed to clay. The relatively high concentrations of radio-phosphorus found in the algal cells demonstrates a movement of the isotope from the clay to the cells.

Of the total ^{32}P adsorbed to the clay particles approximately 30% was assayed in the algal cells, without regard to light regime, after two days. Rigler (1956) showed a similar uptake of ^{32}P from lake water by plankton, stating that the percentage of activity in plankton at the surface (0-2m) reached a maximum of 50% while in deeper water (8-9m) a maximum uptake of 30% was reached in three days. The ^{32}P adsorbed to clay particles in this laboratory study was nearly as available to algal cells as free radio-phosphorus found in the aqueous phase in a natural lake system.

Phosphate-limited P. morum has the ability to rapidly sorb large amounts of radiophosphorus far in excess of that found in the water and in much greater quantities than can be explained by a simple physical equilibrium. Phosphates are strikingly concentrated by Euglena: 100,000X, Volvox: 140,000X, Pandorina: 285,000X, and Spirogyra: 850,000X (Round, 1970). Because many microorganisms have evolved in environments which have very low nutrient concentrations, it may be characteristic for these algae to have mechanisms for the uptake of nutrients from low levels. It may also be possible for some algae to alter the chemical composition of their immediate environment through metabolic activity, affecting the availability of adsorbed phosphates.

A relatively stable equilibrium developed in the cultures after the second day through compensatory changes in the activity of each phase. Initially the clay particles rich in adsorbed ^{32}P released this isotope to the medium and the cells. Kurtz (1945) suggests that as adsorbed forms of phosphate increase in amount their solubility in water increases rapidly. The rapid loss in cellular activity in the 24-hour light regime on the fourth day was correlated with a simultaneous gain in activity by the clay, evidence that the system can reverse itself to maintain equilibrium. Biggar and Corey (1969) noted a similar exchange and showed that subsoil particles low in

phosphorus may absorb this nutrient from over-laying water, reducing the concentration in solution.

The movement of ^{32}P from the clay and its availability to algal cells appears to have been largely governed by pH and chemical equilibria reactions. In general, high phosphate adsorption by clays is favored by a low pH. Stumm and Morgan (1970) state that maximum adsorption of orthophosphates on kaolinite clay occurs at a pH near 3. Ohle (1937) noted that maximum adsorption by sediments takes place at pH 5.9 and there is a decrease above and below this value. MacPherson, et al. (1958) found a pH above 6.5 created a greater increase of phosphorus in the water. The predominant dissolved orthophosphate species over pH range 5-9 are H_2PO_4 and HPO_4, both of which are available to algae cells.

The relationships between total phosphate in solution and pH was determined using the solubility product principle. The dissociation product constants of the two chemical species $Al(OH)_3$ and $AlPO_4 \cdot 2H_2O$ (Variscite) were 33.8 and 22.4 respectively. The ionic strength of the solution, determined from the major nutrients in the Chu-10 medium, was $22.949X10^{-4}M$. The phosphate activity coefficients of 0.949 for H_2PO_4 and 0.811 for HPO_4 were determined by the Debye-Huckel equation (Klotz, 1958). From these values a minimum phosphorus solubility of about 0.349 mg/l was calculated for pH 6.2. At pH 7.0, 7.4,

and 8.0 the phosphorus solubility rapidly increased to approximately 3.12, 14.5, and 169.0 mg/l respectively. A similar trend of the effect of pH on soluble phosphorus concentrations was shown by Lindsay and Moreno (1960). At pH values approaching 8 and higher the tendency for precipitation of phosphorus becomes enhanced and may be related to the formation of $CaHOP_4$.

The distribution of ^{32}P among clay particles, algal cells and in solution was correlated with pH and appears to follow the predicted trend calculated for phosphorus solubility as a function of pH in the cultures. As the pH increased from the initial pH 6.2 the activity in solution, as well as the activity in the cells increased reaching maximum levels at pH 7.4-7.8. It should be noted that lake sediments and most mineral-bearing waters are known to lie generally within a pH range 6-9, while agricultural soils are characteristically more acidic, pH<6. Significantly, the algal cells by altering the pH, at least partly determine the availability of adsorbed ^{32}P. The greatest uptake of radiophosphorus occurred in the 24-hour light regime where photosynthetic activity was maximized and the pH reached 7.8. This condition was predicted, on the basis of pH and equilibria reactions, to provide the greatest availability of phosphorus.

Numerous investigators have substantiated that the removal of oxygen from natural systems will allow the

release of inorganic phosphorus from bottom sediments (Mortimer, 1941-42; Hutchinson, 1957). A similar trend was described in this study, as the greatest release of ^{32}P from the clay occurred in the cultures maintained in the dark and 12-hour light regimes. These cultures evidenced the lowest concentrations of dissolved oxygen, although oxygen was present at all times. Fitzgerald (1970) determined that phosphorus-limited Selenastrum and Cladophora did not respond by growth when exposed to as much as 2 mg of phosphorus as lake muds under aerobic conditions. Golterman, et al. (1969) reported Scenedesmus obliquus was able to increase tenfold or more with phosphorus from lake muds obtained from aerobic zones. This study indicates that adsorbed phosphates are available to P. morum although increases in growth rate were not evidenced. It is likely that the differences reported may have been due to testing procedures, characteristics of the mud samples, or requirements of the individual algae studied.

In view of the vast reservoirs of phosphates absorbed to the sediments in natural waters, the proportion of phosphorus shown available to algal cells in this study is biologically significant. Phosphorus availability was largely controlled by pH and equilibria reactions and to a lesser degree by oxygen concentrations; while in natural waters with a substantial fraction of

iron present oxygen concentrations may play a major role in determining phosphorus availability.

Increased emphasis and further research is needed in examining the role of adsorbed nutrients in the eutrophication of aquatic systems. General removal of soluble phosphorus at waste-water treatment plants is not the total solution if adsorbed phosphates from other sources are readily available to aquatic plants. Continued effort should be made to understand the modes of nutrient losses and the effects of chemical gradients, pH, and other parameters on the availability of adsorbed forms of phosphorus.

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APPENDICES

APPENDIX A

EQUATIONS FOR CORRECTION OF COUNTER DEAD TIME AND RADIOACTIVE DECAY

APPENDIX A

EQUATIONS FOR CORRECTION OF COUNTER DEAD TIME AND RADIOACTIVE DECAY

From the counted dead time ($T = 1.5928 \times 10^{-6} cpm^{-1}$) a true count rate was calculated using the following equation from Chase (1959):

$$N = \frac{n}{1 - nT}$$

where N = true count rate,
n = observed count rate, and
T = counter dead time.

Because ^{32}P has a half-life of 14.3 days, radioactive decay represented the greatest correction factor. Time of counting was recorded for each sample and all samples were corrected to the time the tracer was added to the cultures by the following formula:

$$\frac{A_t}{A_o} = e^{-0.693 \frac{t}{T}}$$

where A_o = initial activity,
A_t = activity remaining after t,
t = elapsed time, and
e = base of natural logrithms.

APPENDIX B

MEAN ACTIVITY IN *P. morum* FOR EACH LIGHT REGIME DURING THE FOUR EXPERIMENTAL DAYS WITH STANDARD ERROR AND COEFFICIENT OF VARIATION

APPENDIX B

TABLE B-1.--Mean Activity in *P. morum* for Each Light Regime During the Four Experimental Days With Standard Error and Coefficient of Variation.

Day	Photoperiod (hrs)	Activity (cpm)	SE	$\frac{S(100\%)}{\bar{x}}$
I	24	1849	44.91	4.20
	12	1673	45.54	4.71
	0	1309	30.12	3.98
II	24	2567	44.68	3.01
	12	2364	31.44	2.30
	0	2215	25.96	2.03
III	24	2937	67.11	3.95
	12	2555	47.03	3.18
	0	2401	50.53	3.64
IV	24	2224	36.11	2.81
	12	2809	49.73	3.06
	0	2504	30.28	2.09

APPENDIX C

ONE-WAY ANALYSIS OF VARIANCE OF THE EFFECTS OF LIGHT ON THE UPTAKE OF ^{32}P BY ALGAL CELLS FOR EACH EXPERIMENTAL DAY

APPENDIX C

TABLE C-1.--One-Way Analysis of Variance of the Effects of Light on the Uptake of ^{32}P by Algal Cells For Each Experimental Day.

Day	Source of Variation	df	SS	MS	$F_{.01}$
I	Among Photoperiods	2	456279	228139	45*
	Error	6	29982	4997	
	Total	8			
II	Among Photoperiods	2	187612	93806	25*
	Error	6	21952	3658	
	Total	8			
III	Among Photoperiods	2	456936	228468	24*
	Error	6	55614	9269	
	Total	8			
IV	Among Photoperiods	2	525978	262989	20*
	Error	6	78298	13049	
	Total	8			

APPENDIX D

MEAN ACTIVITY IN CLAY PARTICLES FOR EACH LIGHT REGIME DURING THE FOUR DAYS WITH STANDARD ERROR AND COEFFICIENT OF VARIATION

APPENDIX D

TABLE D-1.--Mean Activity in Clay Particles for Each Light Regime During the Four Experimental Days With the Standard Error and Coefficient of Variation.

Day	Photoperiod (hrs)	Activity (cpm)	SE	$\frac{S(100\%)}{\bar{X}}$
I	24	5934	65.35	1.90
	12	5926	117.19	3.42
	0	5785	83.56	2.50
II	24	5417	152.15	4.86
	12	5436	178.70	5.69
	0	5124	65.85	2.22
III	24	5223	152.11	5.04
	12	5398	171.19	5.49
	0	5091	100.38	3.41
IV	24	5728	68.88	2.08
	12	5349	129.41	4.18
	0	5063	89.72	3.06

APPENDIX E

TWO-WAY ANALYSIS OF VARIANCE OF THE EFFECTS OF LIGHT AND TIME ON THE LOSS OF ADSORBED ^{32}P FROM CLAY PARTICLES WITH DUCAN'S MULTIPLE RANGE TEST FOR MEAN SEPARATION

APPENDIX E

TABLE E-1.--Two-Way Analysis of Variance of the Effects of Light and Time on the Loss of Adsorbed ^{32}P From Clay Particles With Ducan's Multiple Range Test for Mean Separation.

Source of Variation	df	SS	MS	$F_{.001}$
Between Days	3	2266324	755441	17.0*
Between Photoperiods	2	665525	332763	7.5*
Interaction	6	369683	61614	1.3
Error	24	1058385		
Total	36			

5933 5926 5785 5728 5436 5416 5398 5349 5223 5124 5091 6063

APPENDIX F

MEAN ACTIVITY IN CULTURE MEDIUM FOR EACH LIGHT REGIME DURING THE FOUR DAYS WITH STANDARD ERROR AND COEFFICIENT OF VARIATION

APPENDIX F

TABLE F-1.--Mean Activity in Culture Medium for Each Light Regime During the Four Days With Standard Error and Coefficient of Variation.

Day	Photoperiod (hrs)	Activity (cpm)	SE	$\frac{S(100\%)}{\bar{x}}$
I	24	71.66	3.38	8.17
	12	73.33	9.82	23.19
	0	120.66	3.28	4.71
II	24	73.00	3.51	8.33
	12	72.00	7.57	18.21
	0	134.0	8.72	11.26
III	24	104.00	2.08	3.46
	12	94.33	11.78	21.62
	0	202.00	7.00	6.00
IV	24	117.33	5.04	7.44
	12	107.66	7.33	11.79
	0	276.00	5.51	3.45

APPENDIX G

CORRELATION MATRIX OF INTERCORRELATIONS FOR ELEVEN PARAMETERS MEASURED

APPENDIX G

TABLE G-1.--Correlation Matrix of Intercorrelations for Eleven Parameters Measured.

	1	2	3	4	5	6	7	8	9	10	11
1	1.000	0.164	-0.529*	-0.629*	0.518*	-0.119	-0.127	0.306	0.241	0.038	0.005
2	0.164	1.000	-0.601*	0.257	0.725*	-0.242	-0.374*	0.008	0.002	0.436*	0.620*
3	-0.529*	-0.601*	1.000	0.363*	-0.512*	0.116	0.040	-0.151	-0.155	-0.033	-0.127
4	-0.629*	0.257	0.363*	1.000	0.000	-0.257	-0.351*	-0.259	-0.255	0.578*	0.713*
5	0.518*	0.725*	-0.512*	0.000	1.000	-0.422*	-0.476*	0.036	0.000	0.664*	0.599*
6	-0.119	-0.242	0.116	-0.257	-0.422*	1.000	0.833*	-0.086	-0.022	-0.393*	-0.399*
7	-0.127	-0.374*	0.040	-0.351*	-0.476*	0.833*	1.000	-0.089	-0.047	-0.509	-0.513*
8	0.306	0.008	-0.151	-0.259	0.036	-0.086	-0.089	1.000	0.947*	-0.140	-0.200
9	0.241	0.002	-0.155	-0.255	0.000	-0.022	-0.947	0.047	1.000	-0.170	-0.238
10	0.038	0.436*	-0.033	0.578*	0.664*	-0.393*	-0.509*	-0.140	-0.170	1.000	0.859*
11	0.005	0.620*	-0.127	0.713*	0.599*	-0.399*	-0.513*	-0.200	-0.238	0.859*	1.000

Note: 1 = Activity in medium
2 = Activity in algae
3 = Activity in clay
4 = Photoperiods
5 = Days
6 = Dry weight algae
7 = Organic weight algae
8 = Dry weight clay
9 = Organic weight clay
10 = pH
11 = Dissolved oxygen
* = Significant correlation (.05 level 0.325)
(.01 level 0.418)