EFFECT OF SUSPENDED SEDIMENT ON THE AVAILABILITY OF ADSORBED PHOSPHOROUS AND IRON TO A GREEN ALGA

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ABSTRACT

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This research concerns several important algal-nutrient-sediment relationships. Scenedesmus quadricauda, a planktonic green alga, was cultured with phosphorous being supplied in soluble and sediment-adsorbed forms. Kaolinite clay was used to typify suspended sediment. Phosphorous-kaolinite was prepared through a series of ion exchange reactions. Growth chambers were specially designed for the experiments.

Scenedesmus quadricauda utilizes adsorbed phosphorus both indirectly and directly from the clay particles. Algae grew extremely well with P-clay as the only source of phosphorous. Besides making phosphorous available to phytoplankton, suspended sediment also increases the availability of iron when the pH of the medium is below 7.5, a condition which normally causes iron to precipitate as ferric hydroxide.

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INTRODUCTION

Sediment transported in runoff water is the most significant source of suspended and deposited sediment in aquatic systems. Suspended solid loads delivered to streams and lakes as sediment in surface runoff are equivalent by weight to more than 700 times the load from sewage (Johnson and Moldenhauer, 1970). The suspended solid load carries with it a vast array of nutrients which are ultimately deposited in lakes. Many investigators have shown that surface drainage, especially from agricultural lands, contains high amounts of phosphorous (Bernhardt et al., 1970; Engelbrecht and Morgan, 1961; Gilchrist and Gillingham, 1970; Keup, 1968; Romkens and Nelson, 1974). Increased concentrations of inorganic phosphate entering a natural water system are extremely important in that phosphorous is a critical nutrient for algae and is most often the element limiting algal growth. After the sediments are deposited, they take on a very important role in regulating the phosphorous cycle in natural water systems. The phosphates in the sediments may be capable of supporting algal growth for some time even if all inputs of phosphates to the system are eliminated. Sediments are frequently capable of sorbing large amounts of added inorganic phosphorous (MacPherson et al., 1958; Harter, 1968; Shukla et al., 1971; Williams et al., 1970). The phosphorous may be held temporarily and subsequently released to growing macrophytes and

algae. Pomeroy et al. (1965) have shown that the release of exchangeable sediment phosphate to the water is of sufficient magnitude to sustain the growth of phytoplankton. These workers also suggested that the development of algal blooms in many natural waters depend upon phosphates from the sediments. Several investigators have obtained excellent growth of Scenedesmus spp. in cultures where mud was the only source of phosphorous (Golterman et al., 1969; Chiou and Boyd, 1974).

In well oxygenated lakes, there exists an oxidized microzone at the sediment-water interface which acts as a barrier against diffusion of phosphates from the mud to the water (Mortimer, 1941). Breakdown of the microzone is brought about by turbulence in the waters which promotes particle suspension. McKee et al. (1970) have found that wind induced currents, density currents and wave action are the principal mechanisms for suspending and transporting sediments within a lake.

Suspension of sediments increases their effect on the overlying water. Under completely mixed conditions, relatively rapid release of phosphate occurs under both anaerobic and aerobic conditions from a variety of lake sediments (Lee, 1970). Zicker et al. (1965) found that agitation of the sediments by turbulence almost doubles the rate of phosphate release from the sediments as compared to undisturbed systems. These results point to the importance of mixing processes in determining the relative flux of phosphorous from the sediments. Large amounts of sediments are

mixed into the overlying waters under severe conditions of turbulence and runoff.

The purpose of this research was to determine if algae cultured in suspension with clay particles were able to directly utilize sediment-adsorbed phosphorous. Growth comparisons were made among cultures containing soluble phosphate and cultures containing suspended clay particles as the only source of phosphate. Although not an initial consideration, this research has also shown that suspended sediments assist in the availability of iron.

METHODS AND PROCEDURES

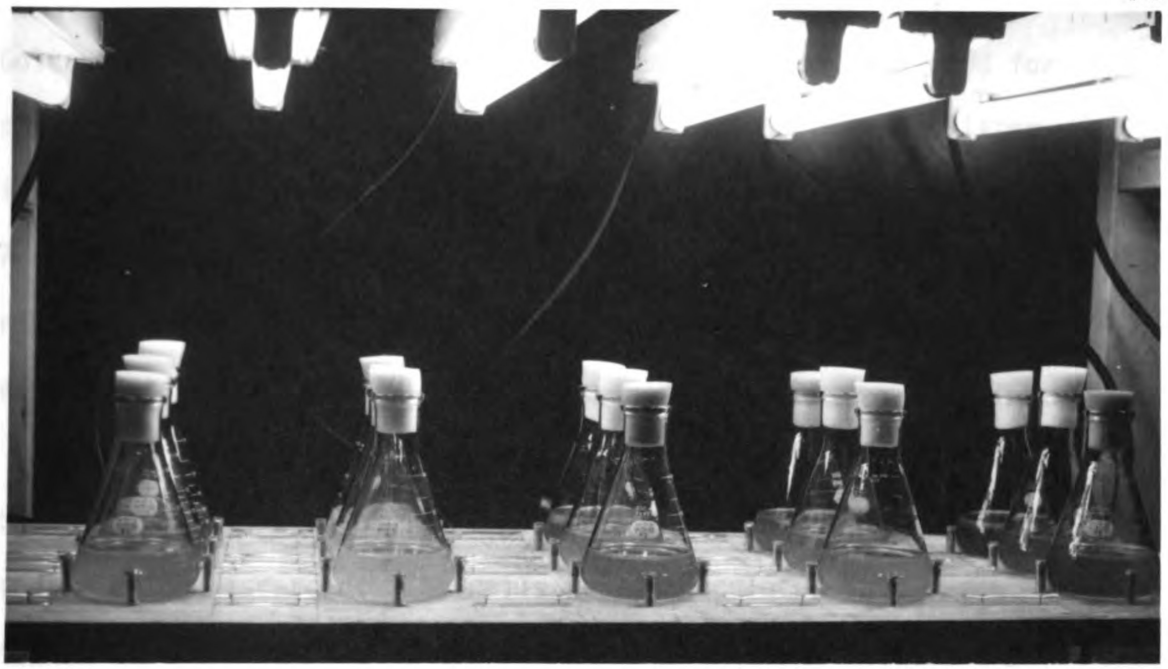
Culture Conditions

All glassware used in the experiments was washed with sodium-dichromate-sulfuric acid solution and rinsed thoroughly with deionized water to assure the absence of phosphorous. To prevent bacteria from contaminating the algal cultures, sterile serological pipettes were used for all algal innoculations, transfers and sampling. Nutrient media and culture flasks were autoclaved for 30 minutes at 17 psi before use. Culture flasks and experimental chambers were stoppered with polyurethane foam plugs to allow for gaseous exchange while keeping out contaminating particles.

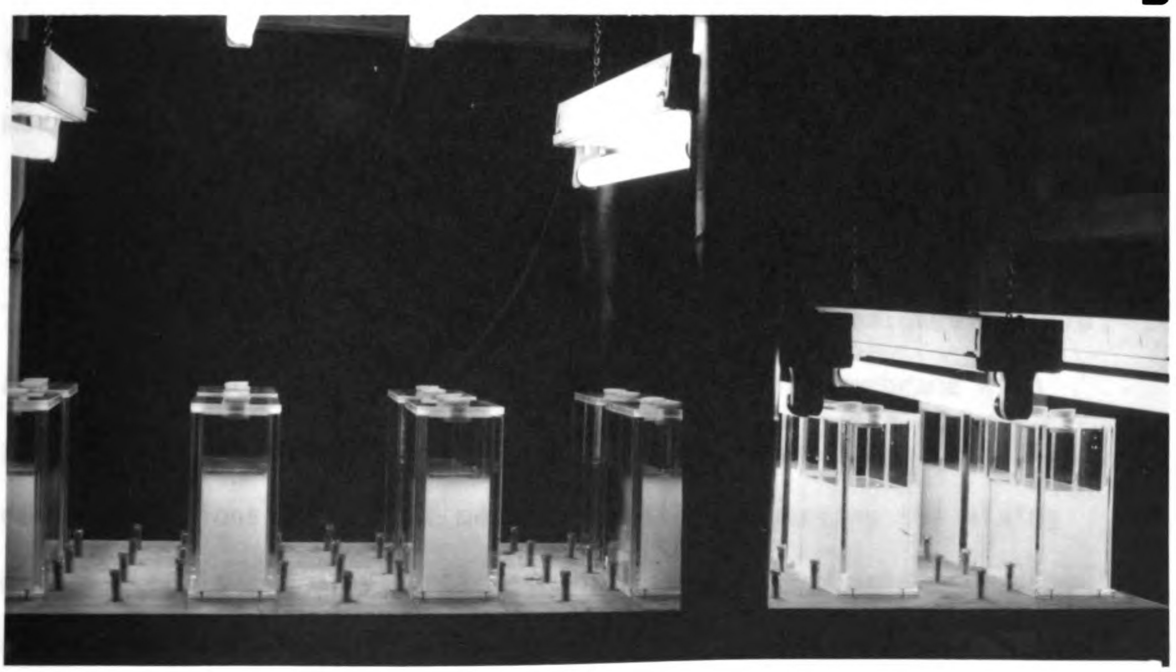
An auxenic culture of the green alga, Scenedesmus quadricauda (614), was obtained from the Indiana University culture collection of algae. The alga was cultured in 300 ml Erlenmeyer flasks in a specially built growth enclosure in which temperature and photoperiod were regulated (Figure 1A). Culture conditions in the flasks were: solution volume, 150 ml; temperature, 25 $\pm$ 2°C; 14 hours illumination per day; light intensity of 125 ft-c provided by Westinghouse Agro-Lite fluorescent lights; continuous agitation of 100 oscillations per minute during the light period; and initially equivalent nutrient concentrations.

Figure 1. Photographs of the growth enclosure containing culture flasks (A) and experimental chambers (B).

A



B



A modified Rodhe VIII culture medium (Rodhe, 1948; Golterman, 1960), with added micronutrient solution, was used for stock cultures and growth experiments (Table 1). One-milliliter innoculates from stock cultures were transferred to new medium at 7- to 14-day intervals to ensure that cultures contained young, rapidly dividing cells which would grow quickly when placed in experimental medium.

Four days prior to an experimental run, a culture of algal cells was centrifuged at 1300 rpm for 5 minutes, the supernatant medium being discarded. The cells were innoculated into fresh nutrient medium containing no phosphorous. During the four-day period, the algae depleted their stored phosphorous and became phosphorous-starved, a condition required for experiments involving phosphorous uptake. Scenedesmus quadricauda cells, when P-starved for periods greater than 4 days, were observed to be in very poor morphological condition.

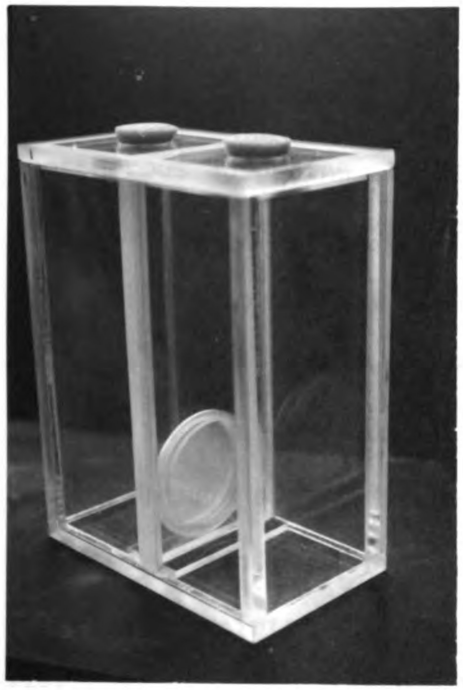
Special chambers constructed of plexiglass were designed for culturing algae under the experimental conditions (Figure 2). The removable center partition of the chambers was designed to hold a membrane filter 47 mm in diameter. A nucleopore membrane with pore size of 3 microns was used because it allowed free movement of nutrient ions across the membrane, while preventing the mixing of algal cells and clay particles which were innoculated into opposite sides of the chamber (Figure 2C). Conditions in the experimental chambers were the same as in the culture flasks except for a larger solution volume (450 ml).

TABLE 1.--Composition of Modified Rodhe VIII Culture Medium (Rodhe, 1948, Golterman, 1960).

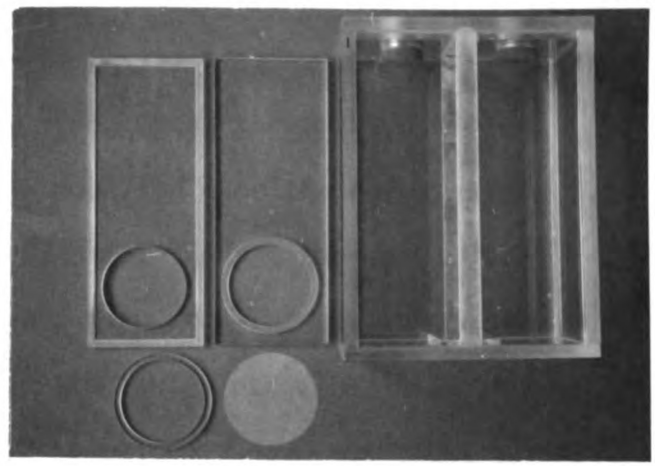
Nutrients	Concentration
Major	mg/l
$Ca(No_3)_2 \cdot 4H_2O$	10.0
$MgSO_4 \cdot 7H_2O$	20.0
K_2HPO_4	5.0
NH_4NO_3	30.0
$NaHCO_3$	80.0
Ferric Citrate	1.0
Citric Acid	1.0
Micronutrients	
H_3BO_3	0.185
$MnCl_2 \cdot 4H_2O$	0.06
$ZnCl_2$	0.01
$CoCl_2 \cdot 6H_2O$	0.002
$Na_2MoO_4 \cdot 2H_2O$	0.01
Na_2EDTA	0.1

Figure 2. Photographs of the experimental chambers showing construction (A,B), separation of algal cells and clay particles by the partition (C) and the three experimental treatments (D).

A

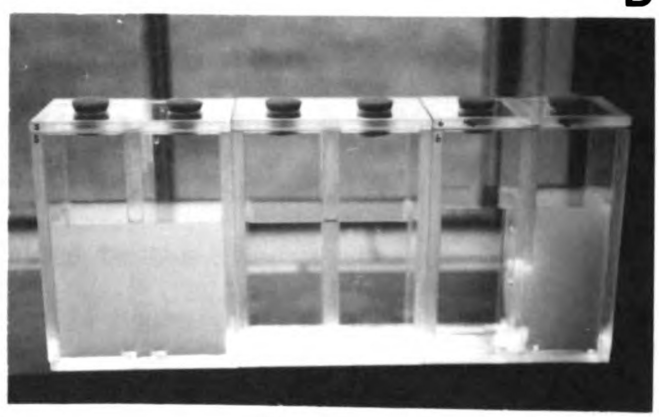


B



C

D



Preparation of P-Kaolinite

Kaolinite, an aluminum clay, was used to typify sediment found in the aquatic system. Many investigators have shown the phosphorous adsorbing capabilities of kaolinite (Stumm and Morgan, 1970; Smith, 1965; Muljadi et al., 1966; Upchurch et al., 1974). The following procedure is slightly modified over the one used by Helfrich (1972) for preparing P-kaolinite. Four liters of a 5% clay suspension were homogenized in a blender. The suspension was passed through a medium porosity IR-120 ion exchange resin which replaced Ca ions with H ions. A 1N solution of NaOH was added to the eluant, maintaining the pH between 10 and 11 and replacing H ions with Na ions. The Na-clay suspension was vigorously shaken and allowed to settle for six hours, after which the clay in solution was siphoned off. The clay which had settled was discarded. The clay suspension was once again passed through the ion exchange column, replacing Na ions with H ions. The kaolinite becomes an acidic aluminum clay which readily adsorbs phosphorous. One gram of phosphate in the form of KH_2PO_4 was dissolved in 1 liter 0.02M HCl. The phosphate solution was added to the clay suspension followed by continuous agitation for 24 hours to insure maximum adsorption. The P-kaolinite suspension was then centrifuged at 8000 rpm for 5 minutes, discarding the supernatant. The P-clay was air dried for 96 hours until a constant weight was achieved, and then stored as a dry powder to retard bacterial growth. Phosphorous analysis of the kaolinite after phosphorous-adsorption revealed a concentration of 373 ug P/gram of clay.

Experimental Design

Twelve experimental chambers were used in a (4 X 3) experiment designed to compare differences in growth attained using different sources of phosphorous (Figure 1B). In the first four chambers, phosphorous was initially available in soluble form, mixed freely with the algal cells. The next four chambers contained phosphorous adsorbed to clay particles as the phosphate source. The algal cells and clay particles were separated by a partition allowing no physical contact between the two. The remaining four chambers also contained P-clay as the only source of phosphate, but the algae and clay were kept in mixed suspension with no partition present. Figure 2D illustrates each of the three chamber types.

The initial phosphorous concentration in each chamber was 414 ug/liter, whether in soluble or adsorbed form. This concentration was achieved in the chambers containing clay by adding 0.5 gram of P-clay. In the chambers without clay, 1 ml of a solution containing 1.0468 grams K_2HPO_4 per liter of deionized water was added. An appropriate volume of P-starved Scenedesmus quadricauda culture was added to each of the chambers to obtain an initial density of 150 cells per ml of medium. The solution in each chamber was stirred thoroughly for one minute, three times daily, the first being at the onset of the light period to aid the shaker in keeping the solutions in each of the chambers in suspension during the light period.

Measurement of pH and Phosphorous Concentration

Sampling was carried out every other day. At the end of the dark period, the culture chambers were removed from the enclosure and thoroughly stirred prior to sampling. Measurements of pH were taken in each chamber with a Beckman pH meter and recorded to the nearest tenth of a unit. In those chambers containing partitions, pH readings were taken in both algal and clay sides. A 15-ml sample was then pipetted from each chamber, but was taken from the algal side only in the chambers with partitions. Three ml of each sample were placed into small capped vials from which cell counts were taken within two hours. The remaining 12 ml were expelled into 15-ml centrifuge tubes and centrifuged at 2500 rpm for 5 minutes. Ten ml of the clear supernatant were pipetted into clean, dry 125-ml Erlenmeyer flasks for the ascorbic acid method of measuring soluble phosphorous concentrations (American Public Health Association, 1971).

In order to keep from decreasing culture volumes by too large a factor over the experimental period, the recommended 20-ml sample for phosphorous determination was halved. Reagents were also added in one-half the usual amounts. Some preliminary experiments using standard phosphate solutions of varying known concentrations showed that this decrease in volume of sample and reagents, as long as it was kept proportional, did not alter the results of the method.

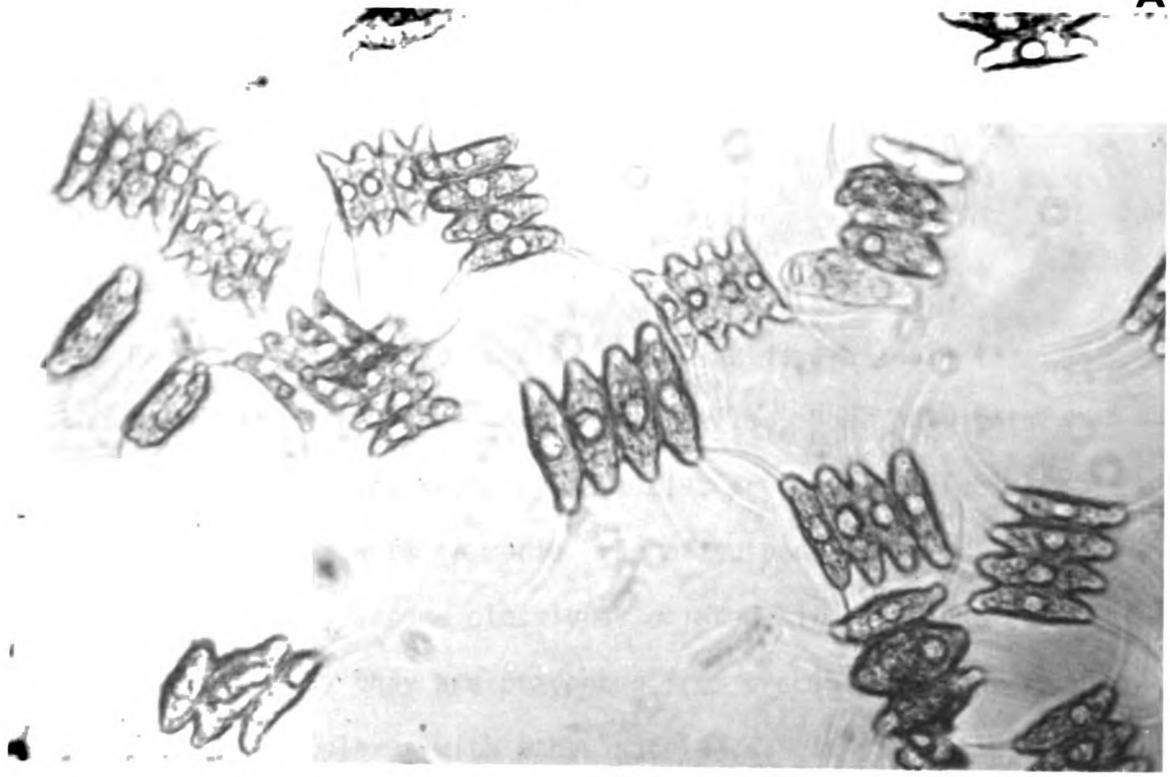
Phorphorous concentrations were measured to determine at what point in time phosphorous in solution reached concentrations limiting to cell growth.

Measurement of Algal Growth

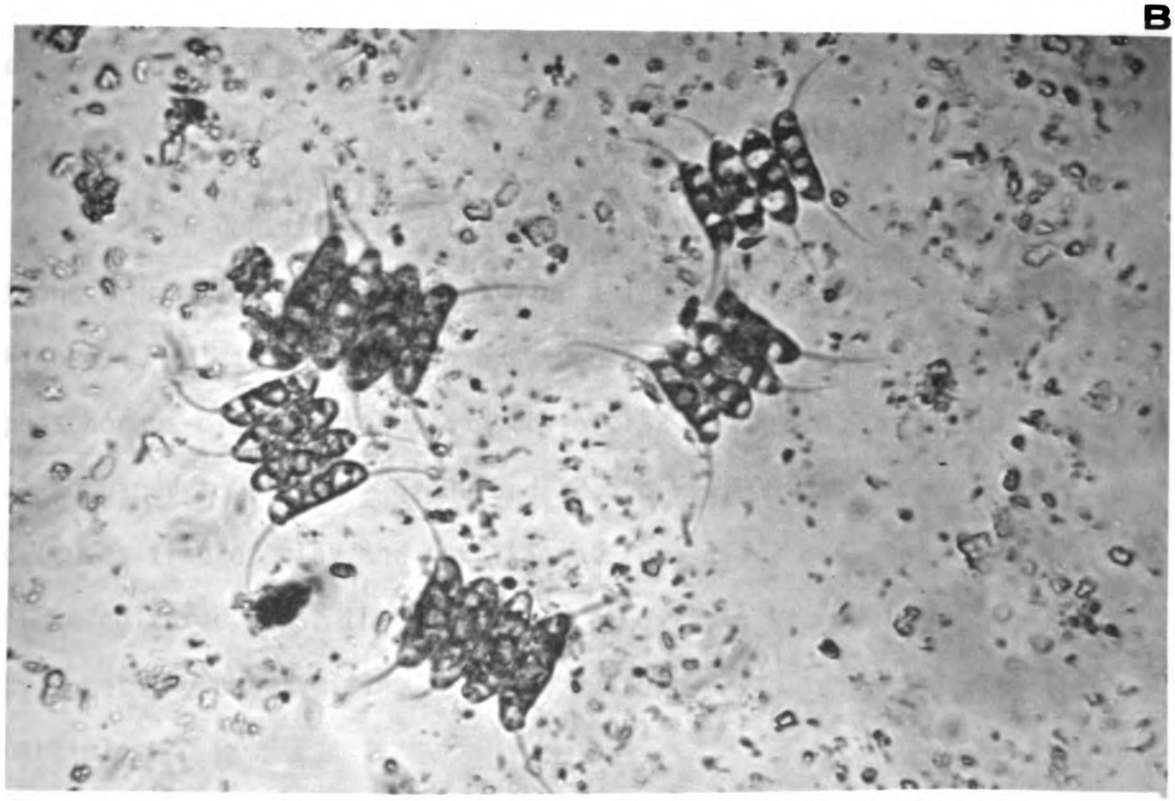
Algal densities were determined using Palmer-Maloney counting chambers and a standard microscope equipped with a Whipple square in the ocular. Counting chambers were loaded by pipette from the 3-ml samples taken at the beginning of the sampling day and allowed to set 10 minutes before counting to facilitate the settling of cells. Cell densities were recorded as algal cells per ml. Figure 3 shows Scenedesmus quadricauda in culture medium free of clay and containing clay.

Figure 3. Photographs of Scenedesmus quadricauda (450X) in culture media free of clay (A) and containing clay (B).

A



B



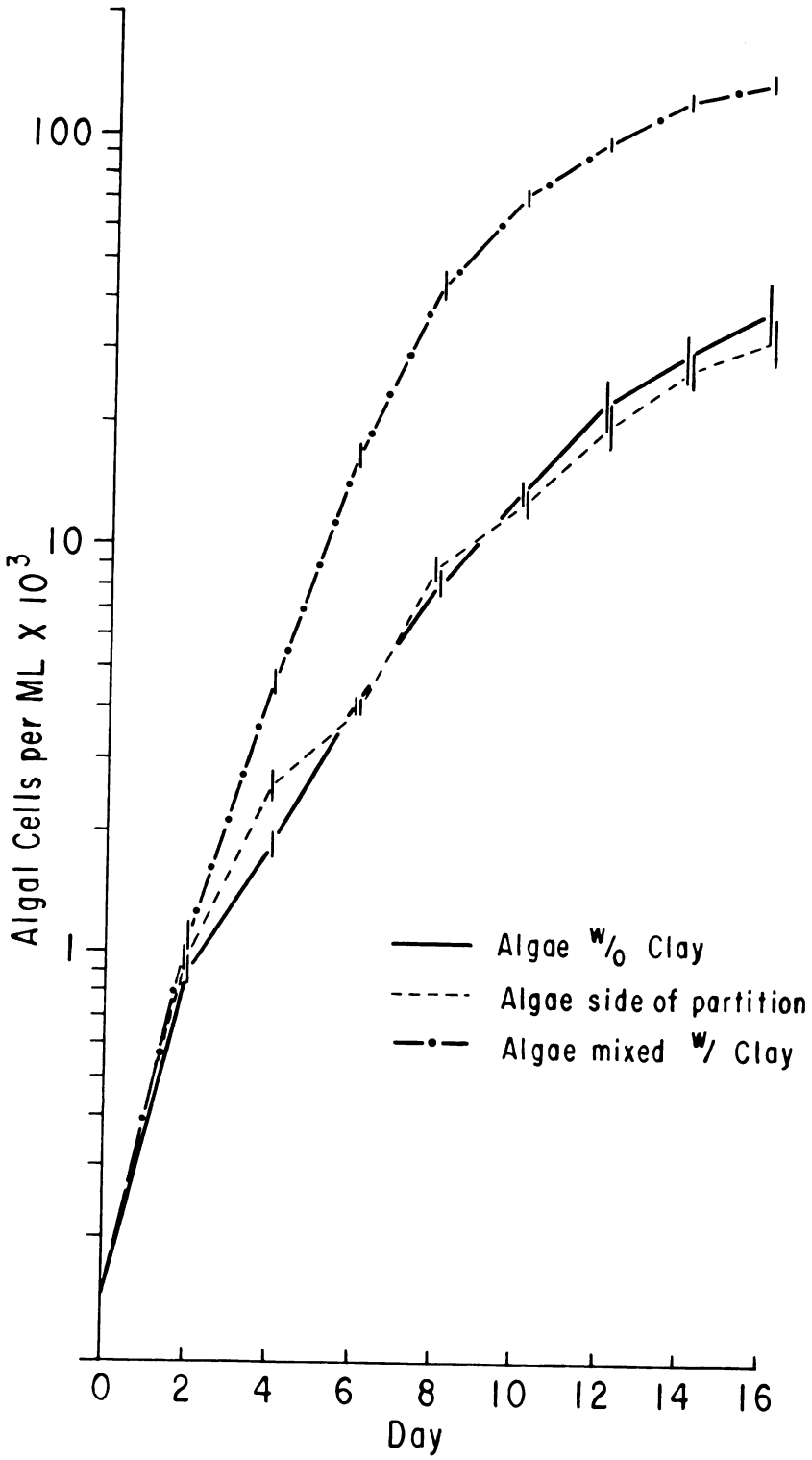
RESULTS

Phosphorous adsorbed to clay particles is available to phosphate-limited Scenedesmus quadricauda cells. Some nutrients in media containing suspended clay particles are more available to algal cells than nutrients in media free of suspended clay. The nutrients in some way become closely associated with or bound to the sediment, whereas they are prevented from precipitating or from forming chemical complexes with other nutrients. It is the author's hypothesis that algae cultured in a sediment suspension not only utilize dissolved nutrients but also remove the adsorbed nutrients directly from the sediment particles. Suspended sediment tends to enhance nutrient availability.

Densities of Algal Cultures

Mean densities of algal cells cultured under three conditions of available phosphorous over a 16-day period are shown in Figure 4. Algae cultured in a suspension of nutrient media and phosphorous-clay reached a density of 137,000 $\pm$ 5,000 cells per ml as compared to 38,000 $\pm$ 6,000 and 32,500 $\pm$ 4,000 cells per ml in cultures free of clay and partitioned from clay, respectively. Algae cultured under the latter two conditions seemed to be limited by something not limiting to algae cultured with suspended clay particles.

Figure 4. Mean cell densities (± one standard error) of algal cultures over the 16-day experimental period.


100
10
1
Algal Cells per ML X 10^3
0
2
4
6
8
10
12
14
16
Day
Algae w/o Clay
Algae side of partition
Algae mixed w/ Clay

Statistical Analysis of Growth Curves

Growth curve data were statistically handled in a split-plot, repeat-measure design (Dr. John Gill, personal communication). In such a design, a comparison can be made between treatments over a period of time. The growth data of three successive experimental runs were combined for each of the eight sampling days. An 8 X 8 matrix containing sums of products and sums of squares was made for each of the three treatments. These three matrices were combined into one 8 X 8 matrix containing covariances and variances. This matrix was then inverted by computer. A multivariate parallel to the "t-test" was used in determining which treatments were significantly different. The values contained in the inverse matrix were used in calculating test statistics, which were then compared with appropriate critical values provided by Dr. Gill.

As shown in Figure 4, algae cultured in suspension with clay particles attained significantly greater densities at a 99% confidence level than algae cultured in media free of clay and separated from clay. Statistical analysis also showed that algae cultured without clay expressed significantly greater growth at a 99% confidence level than algae partitioned from the clay. This difference is not apparent in Figure 4, probably due to the logarithmic scale used in plotting data points.

Soluble Phosphorous Concentrations

Mean concentrations of soluble phosphorous in the algal cultures over the 16-day experimental period are shown with their

standard errors in Figure 5. Soluble phosphorous concentrations lower than 20 ug/liter are limiting to growth of Scenedesmus quadricauda (Rodhe, 1948). Algae cultured in media free of clay and separated from clay did not become phosphorous-limited over the 16-day period, but did reach limiting concentrations on about day 19 as compared to day 11 in clutures with suspended clay. Phosphorous was being utilized at a much slower rate in cultures not containing clay in mixed suspension. The cultures were being limited by something other than phosphorous.

Relationship of pH to Nutrient Availability

Figure 6 shows pH ranges over the 16-day period for each culture condition. Initially, solutions containing clay were slightly more acidic than solutions free of clay due to the acid character of the P-kaolinite. Algal cultures free of clay and separated from clay showed average pH values of 7.6 over the entire 16-day period. Algae in suspension with clay particles exhibited pH values increasing from 7.7 to 8.7, an indication of increased growth and reduction of CO_2 concentrations during photosynthesis.

Effect of Various Iron Sources on Algal Growth

An experiment was designed to determine the effects of various iron sources on the growth of algal cultures in an attempt to establish if iron had limited growth in the previous experiment. Twelve experimental chambers were established, each with an initial iron concentration of 0.18 mg/liter as in the previous experiment.

Figure 5. Mean concentrations and standard errors of soluble phosphorous in algal cultures over the 16-day experimental period.

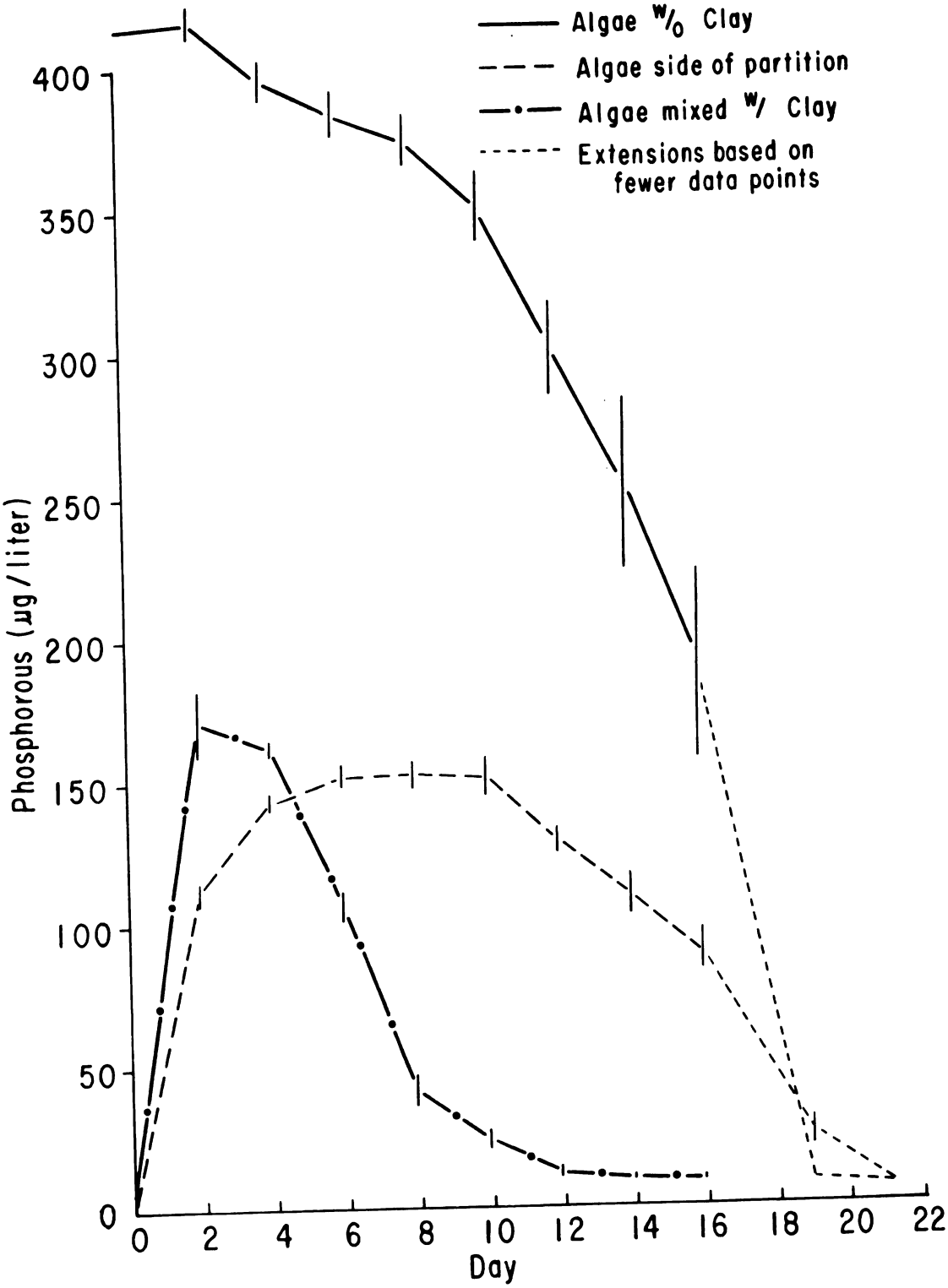
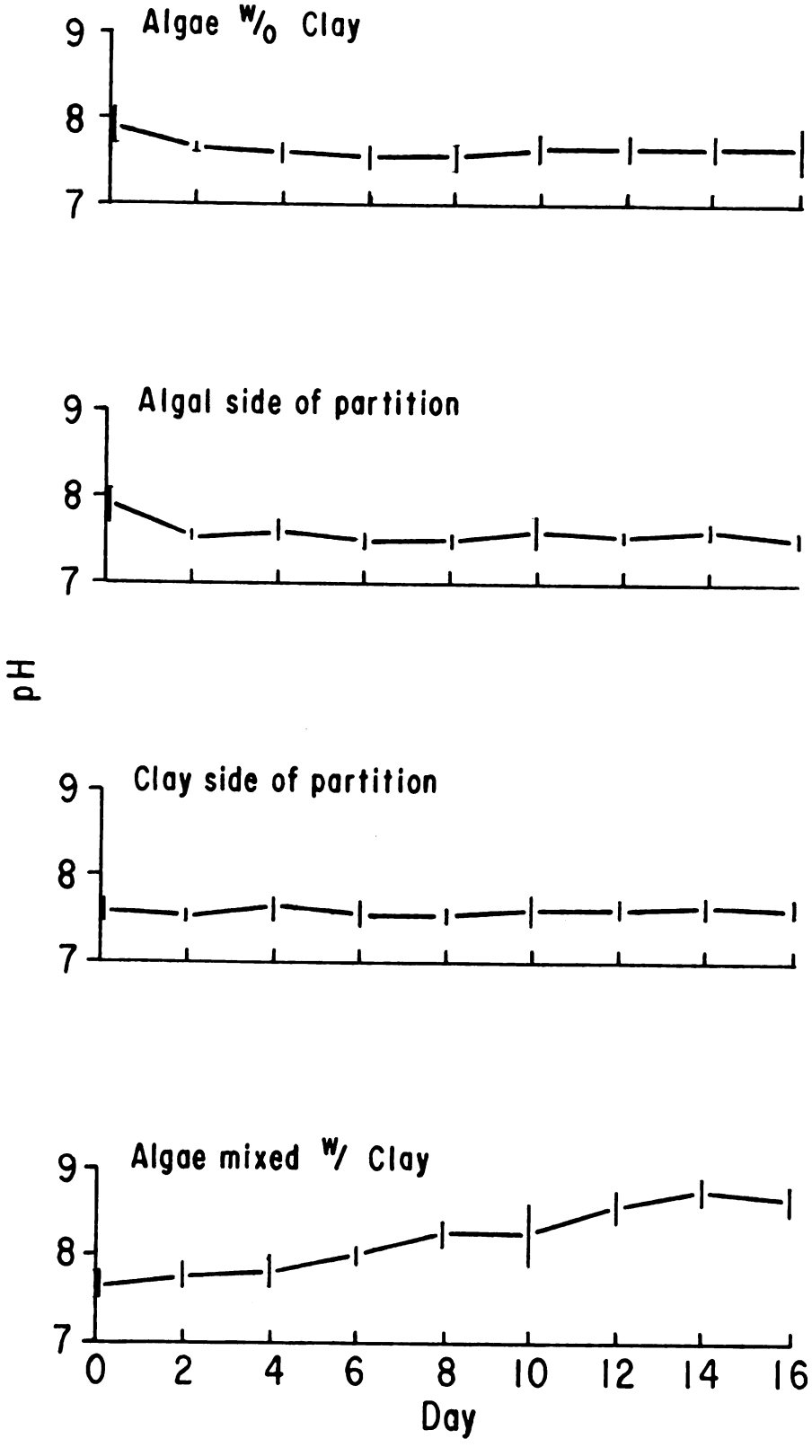

Algae w/o Clay
Algae side of partition
Algae mixed w/ Clay
Extensions based on fewer data points
Phosphorous (µg / liter)
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
Day

Figure 6. Ranges of pH values of algal cultures over the 16-day experimental period.


Algae w/o Clay
Algal side of partition
Clay side of partition
Algae mixed w/ Clay
pH
9
8
7
0
2
4
6
8
10
12
14
16
Day

The way in which the iron was supplied varied among chambers. Four of the chambers contained algae and P-clay in suspension as in the previous experiment, with iron citrate as the iron source. The remaining eight chambers contained an innoculate of algae in nutrient media free of clay. In two of the chambers, initial iron concentrations were supplied by ferric citrate as in the previous experiment. The next two chambers were prepared as the preceding two except that a concentration of iron, equal to the initial concentration, was added every other day as ferric citrate solution. The remaining four chambers were set up with iron being initially supplied as Fe-EDTA in two of the chambers, and Fe-EDDHA in the other two chambers.

Mean algal densities and ranges resulting from the experiment are shown in Figure 7. Ferric citrate, added every other day in concentration equal to the initial amount, produced algal densities slightly greater than densities obtained in the clay suspensions. Fe-EDTA produced algal densities equal to those densities in the clay suspensions. In the chambers where iron was supplied as Fe-EDDHA, algal densities were 20,000 cells per ml less than when clay was present. The lowest cell densities were achieved in cultures provided with only an initial ferric citrate solution.

Mean soluble phosphorous concentrations and ranges of the algal cultures receiving various iron sources are shown in Figure 8. The concentrations of soluble phosphorous decreased in relation to increases in algal densities. Algae cultured in chambers with ferric citrate added every other day experienced phosphorous-limitation on

Figure 7. Mean cell densities and ranges of algal cultures treated with various iron sources over a 20-day period.

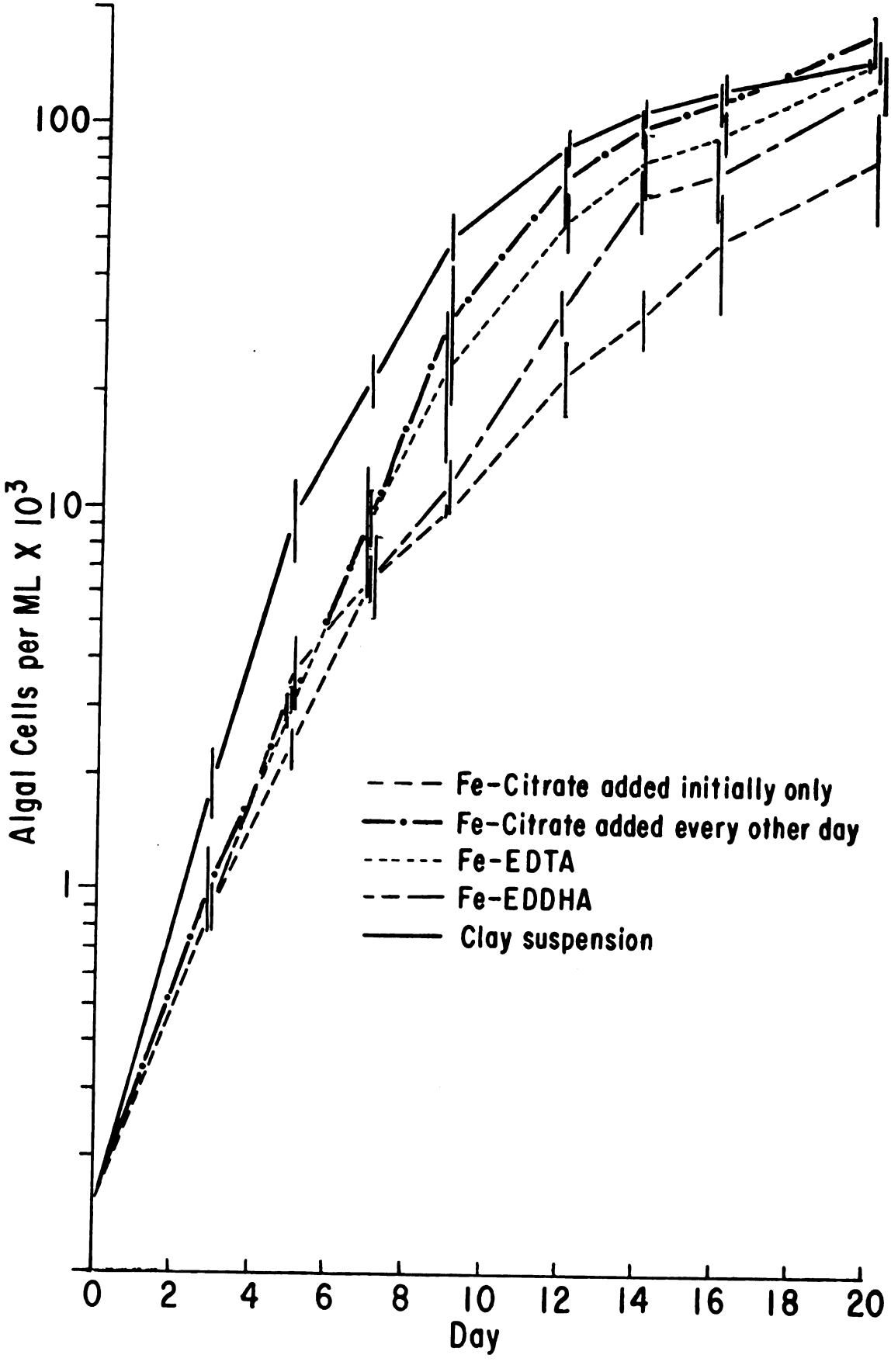
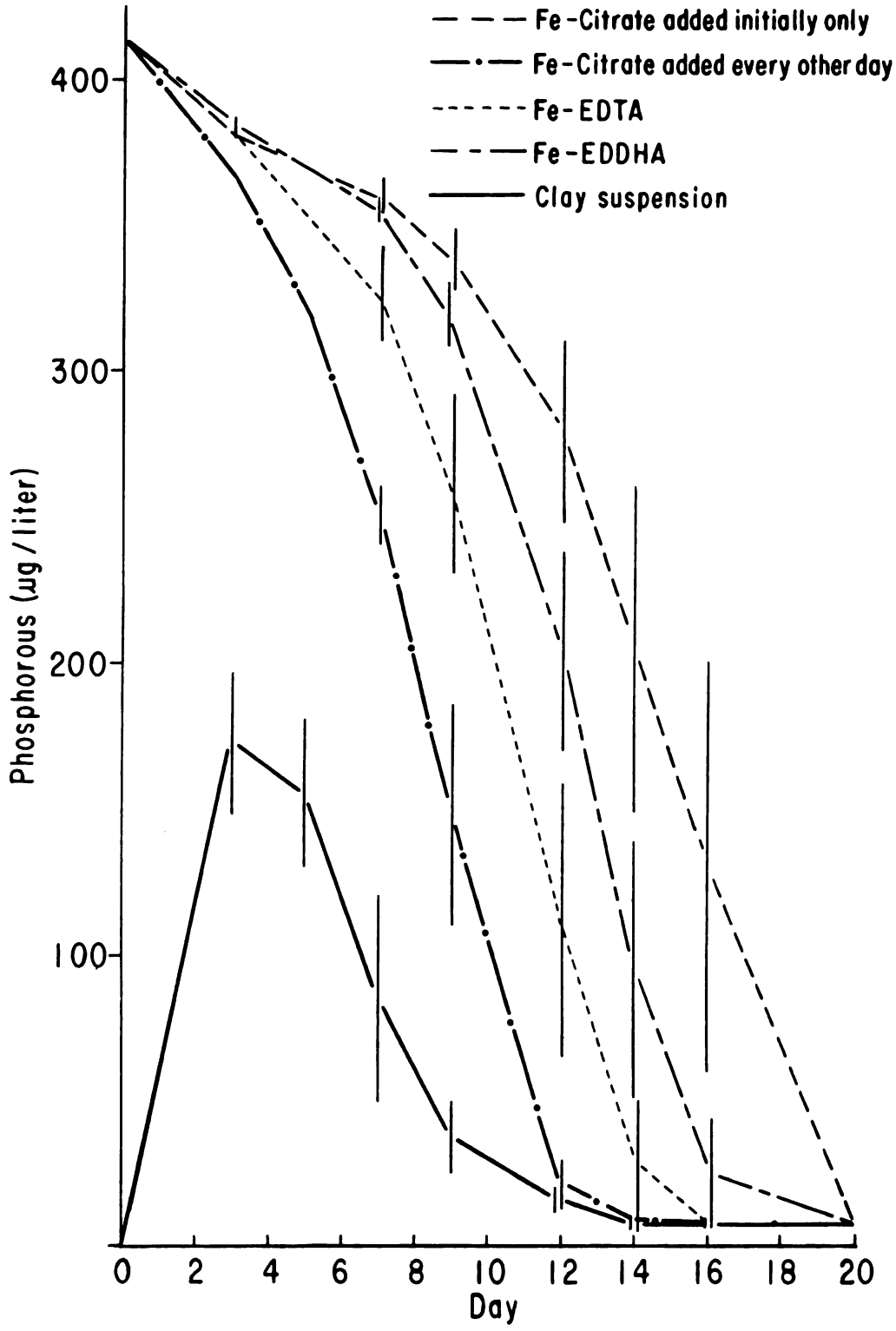

100
10
1
Algal Cells per ML X 10^3
0
2
4
6
8
10
12
14
16
18
20
Day
Fe-Citrate added initially only
Fe-Citrate added every other day
Fe-EDTA
Fe-EDDHA
Clay suspension

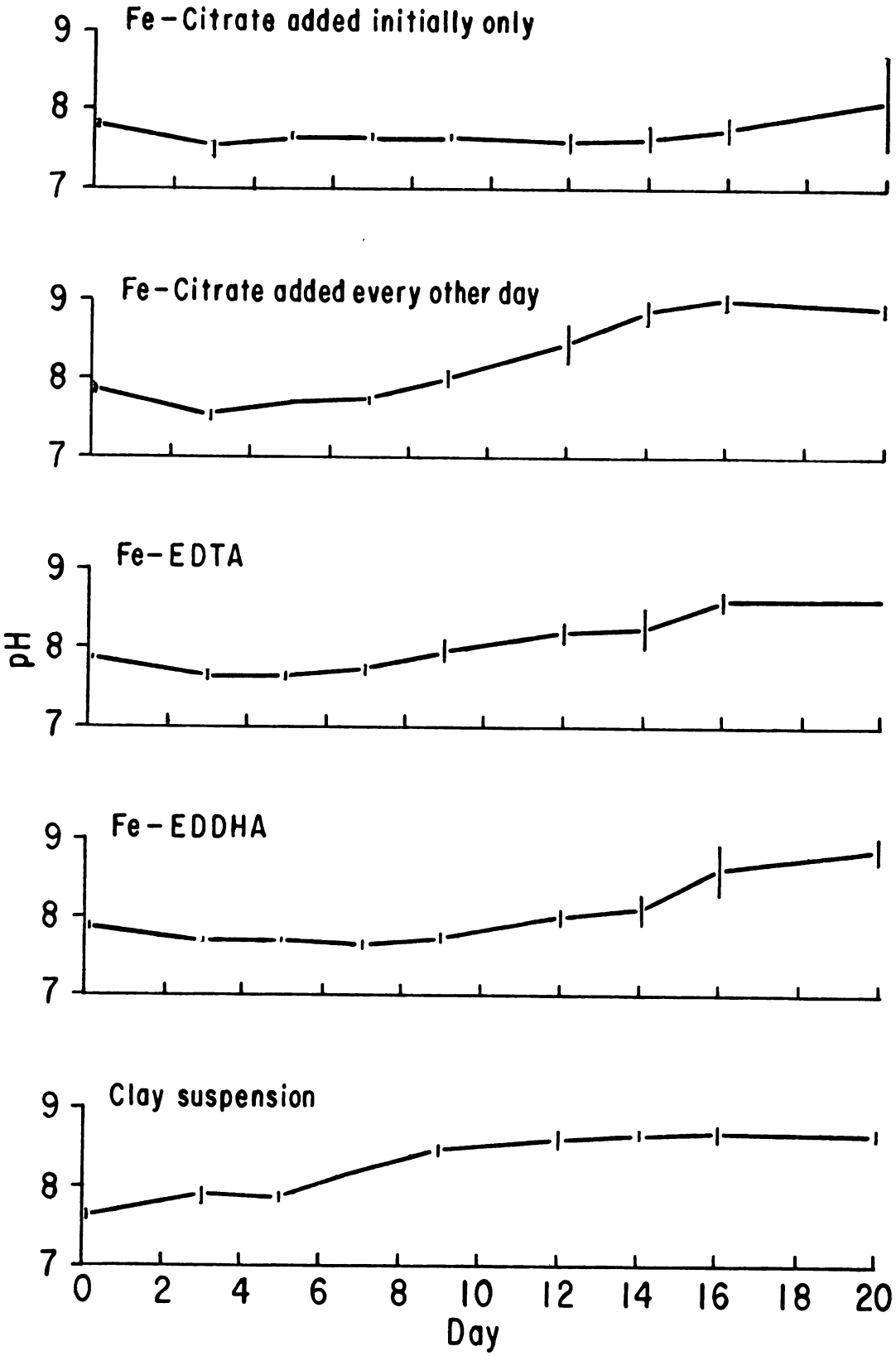
Figure 8. Mean concentrations and ranges of soluble phosphorous in algal cultures treated with various iron sources over a 20-day period.


Fe-Citrate added initially only
Fe-Citrate added every other day
Fe-EDTA
Fe-EDDHA
Clay suspension
Phosphorous (µg / liter)
400
300
200
100
0
2
4
6
8
10
12
14
16
18
20
Day

day 12, as compared to day 11 for algae cultured in clay suspension. Algal cultures supplied with initial concentrations of Fe-EDTA, Fe-EDDHA and Fe-citrate became phosphorous-limited on day 14, day 16 and day 20 respectively.

Ranges of pH values of the algal cultures in the iron source experiment are shown in Figure 9. Cultures with ferric citrate added only initially experienced low pH values over most of the experimental period. In the other four culture conditions, pH values increased to between 8.6 and 9.0. These higher values are due to the increased algal growth and the subsequent reduction in CO_2 by photosynthetic activity.

Figure 9. Ranges of pH values of algal cultures treated with various iron sources over a 20-day period.


Fe-Citrate added initially only
Fe-Citrate added every other day
Fe-EDTA
Fe-EDDHA
Clay suspension
pH
9
8
7
0
2
4
6
8
10
12
14
16
18
20
Day

DISCUSSION

The results of this research have shown that phosphate-limited Scenedesmus quadricauda can utilize this nutrient adsorbed to clay particles. The only source of phosphate initially present in the cultures was adsorbed to clay. The algal cells probably obtain phosphate in two ways: (1) direct utilization off the clay particles; and (2) utilization of dissolved orthophosphate desorbed from the clay. During the first 4 to 6 days of the growth experiments, many of the algal cells were seen to be tightly enveloped by clay particles. This suggests that the P-limited algal cells were somehow concentrating the P-clay particles resulting in phosphate assimilation. The adsorbed phosphate is chemically bonded to positively charged corners and edges of the clay (Al^{3+}) (Stumm and Leckie, 1970; Stumm and Morgan, 1970). Concentrations of the particles around algal cells would bring the phosphate ions in contact with the cells. The long cellular extensions of the Scenedesmus spp. may aid in this process. It may also be possible that the algae are altering the chemical environment around themselves through metabolic activity, increasing the availability of sediment-adsorbed phosphate, by increasing desorption of the phosphate ions.

The algae most likely obtain the bulk of their phosphate supply from dissolved orthophosphate which was desorbed from the

clay particles. Adsorption-desorption of phosphorous is dependent upon pH with maximum adsorption occurring over the slightly acid pH range 4-7 (Murrmann and Peech, 1969). Desorption of phosphorous increases rapidly above and below this range. Stumm and Morgan (1970) found the maximum adsorption by kaolinite to be at a pH near 3. Muljadi et al. (1966) have shown that kaolinite sorption is reversible over a pH range 5-9. The predominant dissolved orthophosphate species of a pH range 5-9 are $H_2PO_4^-$ and HPO_4^{2-}, both of which are readily available to algal cells. Algae are able to increase desorption of phosphate by increasing the pH of the medium through photosynthesizing activity. The pH immediately adjacent to a photosynthesizing algal cell should be higher than the pH in the bulk of the medium. Since the sorption reaction is markedly dependent upon pH, it is probable that the exchange takes place at or near the surface of the algal cell rather than at a distance from the cell. This is another point in defense of the idea that algae obtained adsorbed phosphate directly from the sorption sites.

As shown in the results, algae cultured in medium free of clay and partitioned from the clay suspension became iron-limited in their growth, whereas algae cultured in suspension with clay did not become iron-limited. Citrate can keep ferric iron in solution only up to a pH value of about 7.5, above which the iron precipitates as ferric hydroxide (Stumm and Morgan, 1970). Wetzel (1975) has shown that ferric hydroxide has very low solubility over a pH range 5-8. In the cultures free of clay suspension, the iron is only minimally available due to agitation of the medium.

Suspended clay particles somehow prevent the iron in ferric hydroxide from becoming unavailable, possibly by some physical or chemical bonding to the clay. Ferric hydroxide adsorbed onto clay particles increases the surface to volume ratio of the particles, a condition which tends to keep them in suspension due to increased bouyancy. Wetzel (1975) has indicated that ferric hydroxide is adsorbed onto suspended particles from which iron can be assimilated by phytoplankton. This would explain why the algae cultured in suspension with clay particles did not become iron-limited. In the chambers with partitions, the clay held the iron in an available form in suspension, but the algae were unable to utilize it because of the membrane partition.

Citrate was found to be a very poor iron chelater at pH values greater than 7.5; however, extremely good growth was evidenced in cultures where ferric citrate was added every other day. The reasons for this increase in growth are twofold: (1) iron was added in an initially available form every other day; and (2) the instances of algae coming into contact with iron increased due to the increasing iron concentrations. EDTA (ethylene-diamine-tetraacetic acid) and EDDHA (ethylene-diaminedi-o-hydroxyphenylacetic acid) are chelating agents which bind ferric iron strongly over a pH range 4-9 (Cleton et al., 1963). Cultures containing iron added as Fe-EDTA reached cell densities equal to those reached by cultures containing a clay suspension. Growth in cultures containing Fe-EDDHA was somewhat limited due to a light-limiting effect caused by the red coloration of Fe-EDDHA.

It has been shown that suspended clay particles are just as efficient in keeping iron available as some of the major chelaters in use today. It has also been concluded that phosphate adsorbed to sediments in natural water systems represents an available nutrient source for phytoplankton. These results stress the importance of sediment-adsorbed nutrients to algal growth when sediments are agitated into the water column.

More research is needed on the role sediments play in eutrophication of natural water systems. The key to understanding sediment-nutrient-algae interactions lies in the complexity of the microenvironment surrounding the interactions. A greater emphasis should be placed on research involving the physical-chemical-biological processes being carried out in these microenvironments.

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