MICROBIOLOGICAL INFLUENCES ON PHOSPHORUS RELEASE FROM AEROBIC LAKE SEDIMENTS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

While a universally accepted definition of "eutrophication" does not exist, its symptoms in lakes and reservoirs are widely recognized: decrease in volume due to siltation and sedimentation, increases in the frequency and intensity of algal blooms, succession of biological communities, and declines in economic and aesthetic values of the body of water. The importance of lakes and reservoirs for recreation and as water supplies makes examination and control of the factors which affect eutrophication rates crucial to their continued beneficial use.

One definition of eutrophication that does not rely upon reiteration of its symptoms states that it is the decrease in volume and the increase of stored energy in the lake system. Decreases in volume result from the trapping of allochthonous material, both organic and inorganic, by the lake and from the sedimentation of dead plant and animal material generated within a lake. Organic materials, whether contributed by allochthonous sources or generated within the lake, contain the chemical energy stored in lake systems.

The source of most organic matter generated autochthonously, a source which becomes progressively more significant as eutrophication proceeds, is primary productivity. Increases in primary productivity, symptomatic of increasing eutrophication as well as contributing to the increase in stored energy, depend on the availability of nutrients.

Nutrient availability, particularly of the limiting nutrients phosphorus and nitrogen, is so central to the process of eutrophication that the accumulation of nutrients in lake systems is often taken as a definition of eutrophication.

While nutrient availability is not a component of the previously stated definition of eutrophication, it is nevertheless of tremendous functional importance to the process and is a primary topic of study in the area of lake management. Understanding the factors that control nutrient availability (which understanding would lead to the development of methods to regulate nutrient availability) is the first step toward being able to control the cause of eutrophication instead of treating the symptoms or bearing the costs of the results.

This study was designed to investigate one aspect of phosphorus availability in lakes: the role of selected microorganisms in affecting the release of phosphorus from littoral sediments to the overlying water column. While phosphorus release under anaerobic conditions that develop in high-energy hypolimnions has been widely studied and attributed to be a significant means of phosphorus release from sediments (1, 2), little work has been done to determine what release may be effected from the oxidized surfaces of littoral sediments by the diverse biota living there. Considering: (1) that shallow lakes tend to eutrophy more rapidly than deep ones (3) [reported in (4)]; (2) that shallow lakes have higher ratios of littoral sediment:total benthic sediment surface; and (3) that lake sediments typically contain much greater phosphorus loads than do water columns

above them; evaluation of littoral sediments as a phosphorus source seems in order.

The relative amounts of phosphorus in water and sediment represent the net effects of processes which incorporate phosphorus into the sediments and processes which return sediment phosphorus back to the water column. Realizing that net mass transfer of phosphorus is likely a function of transport rates between, and phosphorus concentrations in, the sediment and water, it is imperative that: (1) the transfer rates themselves; (2) the processes which sustain the transfer; and (3) the conditions which influence the sustaining processes be identified if we are to understand the role of sediments as they influence phosphorus availability in lakes.

The comparatively slow rate of phosphorus transfer out of oxidized sediments is evidenced by phosphorus concentrations in sediments which usually are three to four orders of magnitude higher than phosphorus concentrations in overlying water columns. (See literature review.) Reasoning that chemical processes such as absorption and precipitation are thermodynamically disposed to add phosphorus to sediments in oxidized systems (1, 2), it is hypothesized that some other processes might be operative in phosphorus release. Whereas organically bound carbon, through the mechanism of bacterial metabolism, provides energy for phosphorus release in anaerobic systems, sunlight might provide energy for phosphorus release in aerobic systems through the mechanism of algal metabolism. The reported experiments, therefore, were designed to determine whether algal metabolism could in some way mediate the release of phosphorus from lake sediments in aerobic systems.

LITERATURE REVIEW

The sizeable volume of literature devoted to the significance of sediments as a phosphorus source or trap reflects both the importance of phosphorus to the health of lakes and reservoirs and the importance of sediments in regulating phorphorus distribution. Much of the literature deals with phosphorus concentrations in sediments, cycling of phosphorus between organic and inorganic phases of water and sediment, and physical-chemical influences on phosphorus cycling. In contrast, the biological availability of sediment phosphorus and the effects that biota can have on phosphorus cycling in sediments have received scant attention. Syers et al. (5) and Lee (4) have recently published literature reviews that are concerned with the complex relationships between phosphorus and sediments. The following literature review discusses available information in four areas of concern: (1) Phosphorus Concentrations in Sediment and Water; (2) Cycling of Phosphorus; (3) Physical and Chemical Influences on Phosphorus Exchange; and (4) Microbiological Relationships to Phosphorus Exchange.

Phosphorus Concentrations in Sediment and Water

The potential for sediments to act as a significant source of phosphorus to surface waters is suggested by the relative amounts of phosphorus in sediment and water. Williams et al. (6) reported that

total phosphorus in sediment samples from fourteen hard- and softwater Wisconsin lakes ranged from 674 to 7000 parts per million (ppm) expressed as micrograms of phosphorus per gram of oven dried sediment. Moore (7) reported total sediment phosphorus levels of 778 to 33,238 milligrams per kilogram (equivalent to ppm) for samples taken in river inlets of Lake Erie. Sediments in the lake basin itself averaged 700 milligrams per kilogram.

Total phosphorus concentrations in lake waters are three to six orders of magnitude less than these sediment concentrations. Rigler (8) reported total phosphorus in the water of nine Canadian lakes ranging from 0.005 to 0.113 milligrams of phosphorus per liter (mg-P/l). Hutchinson (9) has summarized data from lakes in several parts of the world and has reported means for unpolluted lake water at 0.01 to 0.04 mg-P/l and for polluted lakes, up to 0.8 mg-P/l.

Cycling of Phosphorus

The correlation between sediment phosphorus levels and the trophic status of lakes is not simple. Sommers et al. (10), for instance, could find no obvious relationship between either total inorganic or total organic sediment phosphorus and the trophic status of several Wisconsin lakes. The complexity of this issue is created by the diverse interactions between lake system components and by the ability of only certain fractions of sediment phosphorus to take place in exchange reactions between solid and aqueous phases in lake sediments.

If significant portions of the phosphorus in lake sediments were available to surface waters, then sediments would provide a

tremendous reservoir of that nutrient for plant growth. In fact, various studies have indicated this to be true. Frink (11) [reported by Stumm and Leckie (12)] has identified a large sediment-phosphate reservoir in a non-calcareous Connecticut lake as being capable of supporting plant growth for a long period even if other sources of phosphorus were not present. Pomeroy et al. (13) estimated that the upper 10 centimeters (cm) of sediment in the estuarine Dobay Sound, Georgia, contained enough exchangeable phosphate to replace the phosphate in solution twenty-five times.

Other authors have taken the opposite view and see lake sediments as a sink for phosphorus, not a source. Fitzgerald (14) grew the green alga Selenastrum capricornutum in aerated tubes with both a 0.02 mg/l PO_4-P defined medium and a similar medium with oven-dried, benthic sediments kept in dialysis tubes as the sole phosphorus source. Algae in the defined medium reached their peak yield in seven days, whereas the sediment-supported algae showed little growth in twelve days. Concurrent experiments showed that sediment from both aerobic and anaerobic areas of his study lakes could sorb orthophosphate under aerobic conditions. Fitzgerald hypothesized from his data that aerobic lake muds could be used to remove phosphorus from lake waters. Olsen (15) [cited by Syers (5)] sees the relative amounts of phosphorus in lake water and sediments as support for the contention that sediments act as a phosphorus sink.

Especially in regard to inorganic phosphorus, sediments have been seen by some (5, 13, 16, 17) to act as both source and sink, i.e., as a "phosphate buffer" (5). Carritt and Goodgall (16) are credited

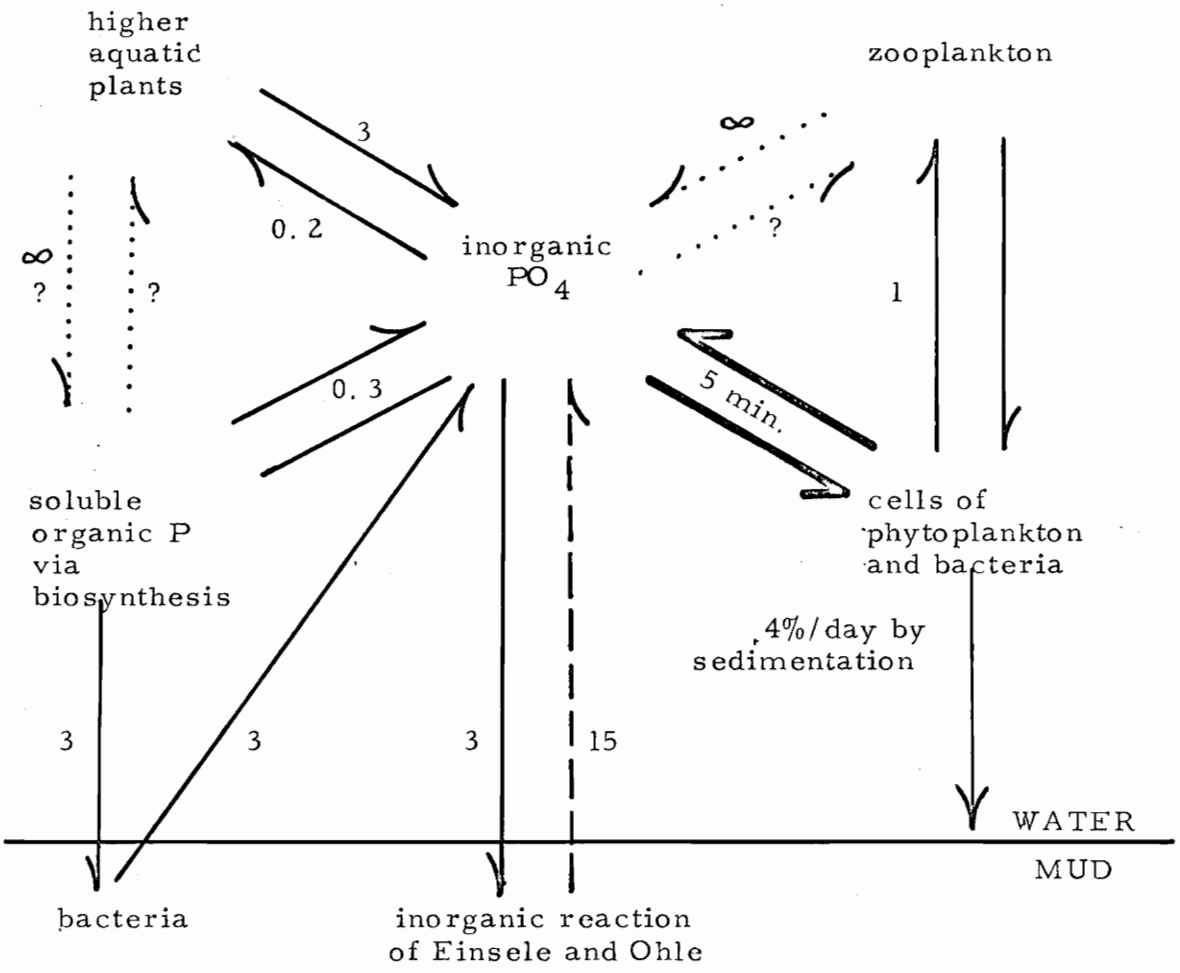
by Pomeroy et al. (13) and by Syers (5) as having suggested this role of sediments based upon their sorption studies on Chesapeake Bay sediments. Harter (17) found that sediments have a large capacity to "intercept and hold large influxes of phosphorus and slowly release it during a period of several days as it may be needed by weeds and algae." He hypothesized that: "If the release is microbial, the sediment may also sorb phosphorus throughout the winter, to be released to the water during the growing season."

Pomeroy et al. (13) concentrated on the concept of exchange to help describe sediment-water phosphorus interactions. They recognized two physico-chemical sorption processes with different rates and a third, biologically mediated, sorption of phosphates in both disturbed and undisturbed estuarine sediments.

Several authors (18) have attempted to diagrammatically represent exchanges between lake system components. Hayes and Phillips (18) presented a diagram of component interactions based on "turnover time," i.e., "the time for as many atoms to move through the phase as are present in the phase." Their diagram is displayed as Figure 1. The diagram is based upon Hayes's and Phillips' work with P^{32} involving inorganic phosphate uptake by bacteria and sediments. Data in this paper include findings of other authors (19, 20) to supplement Hayes's and Phillips' original data.

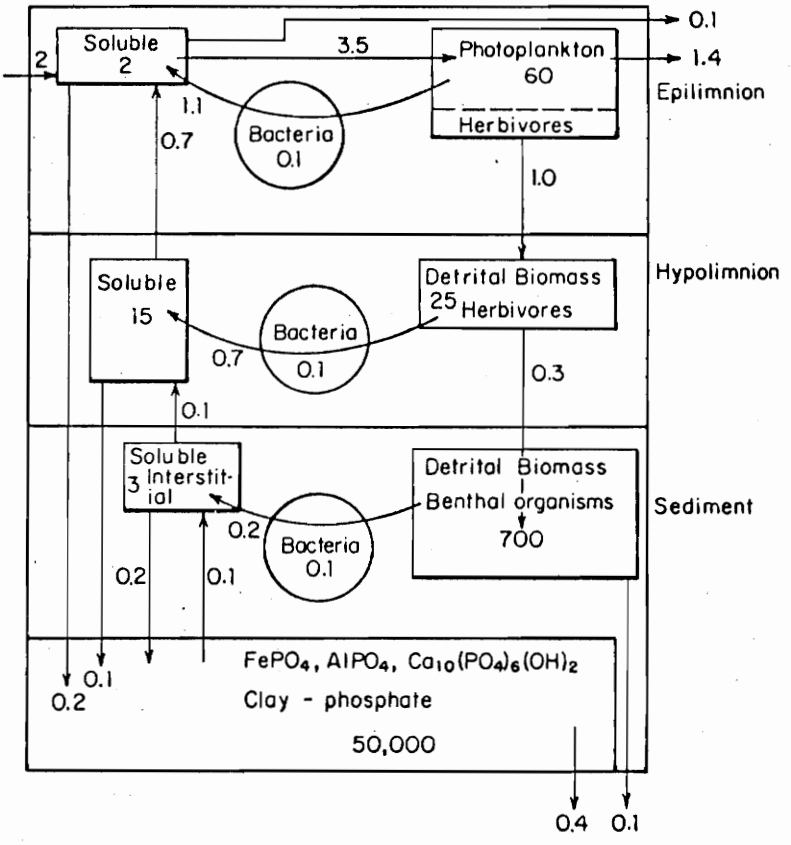
Stumm and Leckie (12) presented a more detailed, but admittedly still simplified, model of phosphorus interactions between lake system components (Figure 2). Instead of turnover rate, which is dependent upon the mass of phosphorus in any component, Stumm expressed phosphorus

Figure 1 - Hayes and Phillips' (18) representation of phosphorus exchanges between lake system components. (Redrawn from (18)).



"TRANSFORMATIONS OF PHOSPHORUS IN A LAKE WITH TURNOVER TIMES FOR DIFFERENT EQUILIBRIA. Very heavy lines indicate the first reaction with floating cells, time in minutes. Other times in days. Lighter solid lines are reactions at intermediate speeds -- two or three orders of magnitude slower than the initial one. Dashed line is the return from mud by inorganic release, a still slower turnover. Dotted lines indicate reactions too slow to measure."

Figure 2 - Stumm and Leckie's (12) model of phosphorus interactions between lake system components based upon exchange rates. (Photostatic reproduction from (12)).



A SIMPLIFIED STEADY STATE MODEL DESCRIBING IMPORTANT STEPS IN THE LIMNOLOGICAL TRANSFORMATION OF P IN A LAKE. The model simulates a real system by giving a hypothetical balance of the abundance of P in various forms (the numbers in the boxes are μg P per liter lake volume) and of the exchange rates (the numbers on the arrows are μg P per liter lake volume per day). The cycle of phosphorus is determined largely by regeneration of P from biota. Primary production depends to a large extent on the supply of P to the trophogenic layer. For deeper lakes the rate of supply from sediments is small in comparison to the supply by the hypolimnion and by the introduction of P from waste and drainage. A significant fraction of P introduced into the lake is irretrievably lost to the sediments.

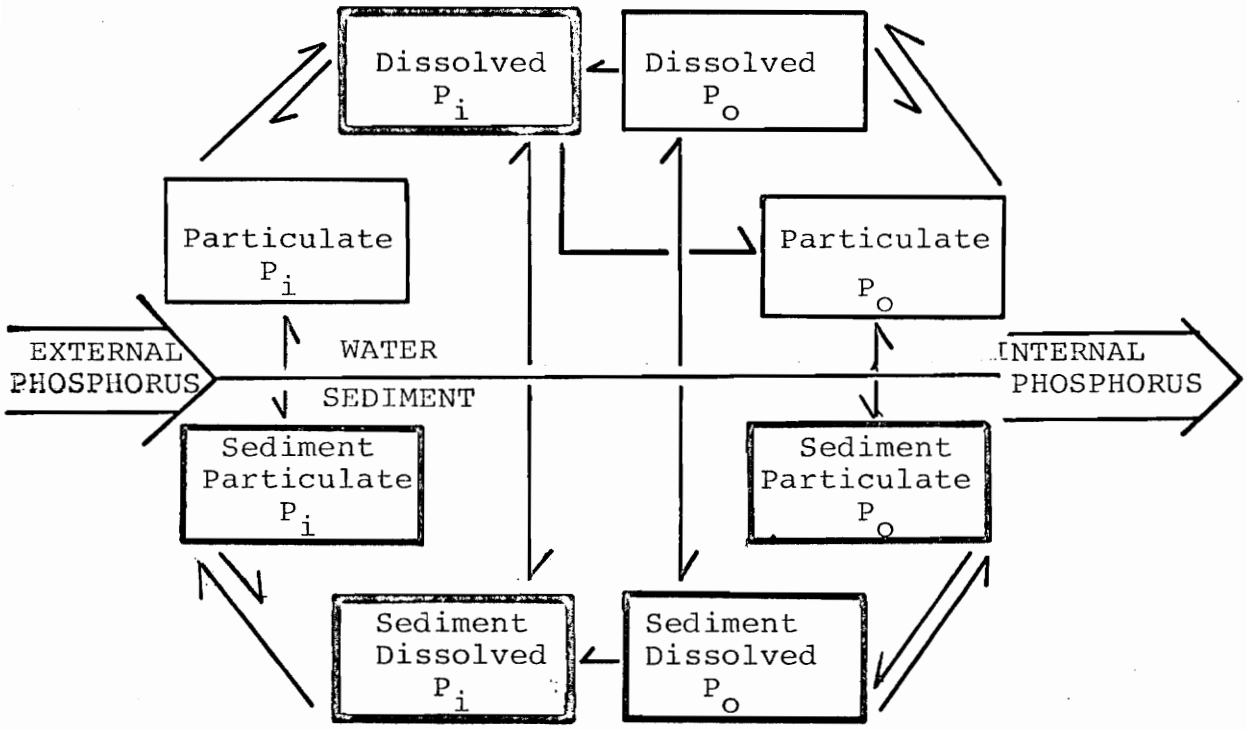
transfer in terms of an "exchange rate" which quantifies a mass transfer rate per unit volume, not per unit of mass of phosphorus potentially available for the transfer. The roles that biotic components play in the transfer of lake phosphorus were given special emphasis.

A third diagram emphasizing sediment-water phosphorus transfer and the importance of soluble inorganic phosphorus in plant availability is shown in Figure 3, reproduced from Syers's (5) literature review. Syers concisely summarized the importance of equilibrium and exchange rates in regard to phosphorus availability in lakes:

> The P cycle in lakes can be considered approximately as a dynamic, steady-state system in which changes in the levels of P in the various compartments are rather small and slow under constant environmental conditions. Interchange of P between components, however, can be extremely rapid . . . even though the P level in the compartments remains unchanged. In assessing the "available P" status of lake systems, information on the rate and extent of interchange among compartments as well as P levels in those compartments are needed.

The diagrams shown in Figures 1-3 are notable for their depiction of the complexity of lake systems. They also are notable for their lack of conceptual similarity, which is surprising since their purposes are very much the same. In June, 1968 the Water Quality Division Committee on Nutrients in Water of the American Water Works Association (21) suggested that problems in understanding and controlling plant nutrients be attacked by considering reservoirs as chemical reactors which are controlled by a number of kinetic processes that might be susceptible to study using systems engineering. Syers's (5) view of phosphorus cycling in lakes as a "dynamic steady-state system" is a step in this direction. The models available in the literature do little more in this direction than illustrate the com-

Figure 3 - Syers' (5) representation of the interchanges occuring between the major P compartments of a lake system. (Redrawn from(5)).



P_i is inorganic P.

P_o is organic P.

plexity of the systems, however.

Chemical and Physical Influences on Phosphorus Exchange

While the need for modeling capability is real, but unsatisfied, a great deal of interest is reflected in the literature in the particular physical, and especially the chemical, factors that affect phosphorus cycling in sediment. Syers (5) has reviewed the literature with the intent of identifying chemical and biological factors which influence the "availability" of sediment phosphorus in lakes. The American Water Works Association's Water Quality Division on Nutrients in Water (21) examined the chemistry of phosphorus and described the physical and chemical forms in which it appears in several environmental settings. Chemical properties of phosphorus also were discussed in relation to their influence on the biological availability of phosphorus.

Syers (5) contended that turbulent mixing is more important than diffusion in affecting phosphorus exchange between sediments and water. Turbulent mixing can increase the depth of sediment which might exchange phosphorus with the overlying water. Estimates of the thickness of surficial sediments which exchange phosphorus range from 1 millimeter (mm) to 15 cm (22).

The availability of phosphorus from sediments even deeper than the few centimeters affected by turbulent mixing may be controlled by diffusion. Hynes and Greib (22) found that P^{32} injected into anaerobic sediment cores 4 cm below the sediment-water interface moved vertically through the sediment in eight days and was released to the overlying water. Approximately the same rate of movement was found in sterile

as in untreated sediment cores, suggesting that the transfer is not biologically controlled. Physical diffusion was suggested as the transfer mechanism.

Temperature and particle settling are physical factors which would have secondary influence on phosphorus availability. Rates of adsorption of phosphorus onto sediments have been shown by Low and Black (23) [cited in Kardos (24)] to be directly related to temperature. This adsorption-temperature relationship might reduce the amount of phosphorus removed from the water column by suspended sediments during the winter. Twenhofel (25) [cited by Syers (5)] found that coarse-textured particles tend to settle in shallow, near-shore areas, whereas fine-textured particles tend to settle in quieter, deep-water areas. Because several phosphorus fractions are closely associated with low-density materials such as clays and organic debris, differential settling of particles might help explain generally higher total phosphorus concentration in deep-water sediments than in shallow-water sediments (26).

In reviewing the ability of soils to "fix," or restrict the mobility in the soil of, phosphorus, Kardos (24) identified three groups of fixation reactions: adsorption, isomorphous replacement, and double-decomposition (precipitation). Isomorphous replacement is said to be the incorporation of the phosphate ion into a crystal lattice following adsorption onto the crystal. Adsorption on a solid is thought to result from the replacement of hydroxyl ions by the phosphate ion. Incorporation into the solid is a much slower and more irreversible process than the adsorption process and may include the replacement of

silica ions as well as hydroxyl ions.

Pomeroy et al. (13) recognized two, strictly physico-chemical processes that typified the "sorption reaction" of phosphate onto estuarine sediments. Like Kardos' explanation of phosphorus "fixation" into soils, the initial process was rapid and the second slower. Pomeroy et al. suggested that "the more rapid of the two sorptive processes is an initial surface sorption and the slower process is a secondary combination of phosphate into the crystal lattice of the clay."

Stumm and Leckie (12) emphasized that the availability of orthophosphate is controlled by the solubility of slightly soluble salts of calcium, iron, and aluminum. These workers found that "heterogeneous equilibria," characterized by the solubility of variscite ($AlPO_4$), strengite ($FePO_4$), and hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$), and the adsorption of phosphate on clays determines the distribution of phosphate between solid and aqueous phases of sediments. The rate determining step for transfer of phosphate from undisturbed sediments is diffusional transport through the sediment's interstitial water. Stumm and Leckie determined a maximum diffusional transfer rate of 0.27 milligrams per square meter per day (mg/m^2 day ^{1}) where the concentration gradient between interstitial and overlying water was steep. They recognized that turbulence and interstitial water displacement by consolidation of sediments may increase the overall transfer of interstitial phosphate to overlying water.

By far the greatest volume of literature on phosphorus in sediments in the past ten years has been generated by a research group at the University of Wisconsin. A 1971 technical completion report (27)

and a review by Syers (5) described the findings of their work. Correlating likelihood of chemical release of phosphorus with the ease with which different sediment inorganic phosphorus fractions are extracted by ammonium chloride (NH_4Cl), ammonium fluoride (NH_4F), sodium hydroxide (NaOH), and acid ammonium oxalate (for non-calcareous sediments) or citrate-dithionate-bicarbonate reagent (for calcareous sediments), the Wisconsin group has applied soil science terminology in labeling the NH_4F and NaOH fractions as "non-occluded" and the reductant soluble fractions as "occluded."

The NH_4Cl fraction represents inorganic phosphorus dissolved in the interstitial water and inorganic phosphorus that is loosely-bound to sediment particles. The NH_4Cl fraction is seen to be chemically "mobile" and, therefore, available to organisms in the sediment or for transfer out of the sediment. The NH_4F and NaOH reagents extract a "short-range order iron (Fe)-rich gel complex" that sorbs phosphate which remains in equilibrium with inorganic phosphorus in solution and is seen as "potentially mobile," i.e., the source for replenishment of interstitial phosphorus. The reductant soluble ("occluded") fraction and calcium hydroxyapatite are considered to be "immobile." The non-occluded, potentially mobile inorganic phosphorus in Wisconsin lakes ranged from 56 to 74 percent of total sediment inorganic phosphorus in calcareous sediments and from 19 to 89 percent in non-calcareous sediments.

Oxidation-reduction potential and pH play important roles in the status of phosphorus in sediments. By affecting solubility products of metal salts and the availability of aluminum, iron, and calcium ions,

pH might determine whether phosphates are precipitated as apatite (at high pH's), sorbed onto ferric or aluminum gels (at low pH's), or precipitated as strengite--$FePO_4 \cdot 2H_2O$--or variscite--$AlPO_4 \cdot 2H_2O$--(at low pH's) (21, 24). The work of Mortimer (1, 2) in relating low oxidation-reduction potentials in sediments to the dissolution of ferric compounds containing phosphorus is well recognized. Chelation of iron and aluminum ions by organic hydroxy acids such as tartaric, citric, malonic and malic acids also has been suggested as a controlling factor in the formation and dissolution of phosphate salts (24, 28).

Microbiological Influences on Phosphorus Exchange

The availability of sediment phosphorus for microbial uptake is indicated by several studies which show that algal and bacterial growth are possible with sediments as the sole source of phosphorus. In 1949, Wohlschlag and Hasler (29) collected sediments from various parts of Lake Mendota. They rinsed their sediments into flasks with distilled water and placed half of the flasks in sunlight, the other half in limited light. After eighteen days, the flasks were shaken and the suspension was microscopically examined and tested for chlorophyll content. All flasks supported algal growth. Darkened flasks supported primarily diatom populations but rapidly developed blue-green algae populations when subsequently placed in the light. The lighted bottles were dominated by blue-green algae populations in which Microcystis aeruginosa and Aphanizomenon sp. were common. Sediments from the river mouths entering the lake supported especially heavy growths and had high proportions of Oscillatoria sp.

Porcella et al. (30) collected surface sediments (top 10-15 cm) from a variety of lakes, placed them untreated in lucite cylinders, and covered them with a defined algal media lacking phosphorus. Some of the cultures were purposely maintained in an anaerobic state by sparging the overlying water with nitrogen. At the end of the experiment, sediments in the anaerobic cultures, as well as those in aerobic cultures, were found to be anaerobic. "Available P" in the sediments, as measured by extraction with dilute fluoride and dilute acid, was correlated with biomass generated in the cultures as measured by volatile suspended solids. The correlation was positive for all sediments. Linear regression of the values for four of the five sediments showed that the total biomass produced increased linearly with the initial available phosphorus. From 60 to 94 percent of the available phosphorus was removed from the sediments in 164 to 209 days using a fill-and-draw sampling routine that gave a ten-day mean residence time for the medium. While the percentage removal of available phosphorus was nearly the same for anaerobic and aerobic cultures during the entire experiment, rates of phosphorus removal were higher in the aerobic cultures. No algae grew in the anaerobic cultures. The more rapid removal of phosphorus was taken as proof that algae act as a phosphorus sink, thereby facilitating phosphorus release from the sediments. The algal succession in the aerobic cultures started with pennate and centric diatoms and progressed to heavy growths of Oscillatoria, despite the fact that all cultures were inoculated with Selenastrum capricornutum, a green alga. Purple sulfur bacteria were found to grow under the Oscillatoria mats. The Oscillatoria population was considered by

the authors: (1) to have been the source of organic materials that provided bacteria with an energy source for maintaining low redox potentials; and (2) to have aided in mixing of the sediments with the overlying water when the algal mat and attached sediment broke loose and floated up through the water column.

Wildung and Schmidt (31) found that algae were able to utilize phosphorus released from sediments held in cellulose acetate dialysis tubing. Twelve percent of the phosphorus associated with the solid phase of their sediments diffused through the tubing in ninety days and then was available for algal growth. This result would seem to argue against Fitzgerald's (14) finding under similar experimental conditions that algae can not utilize phosphorus from aerobic sediments. However, Fitzgerald incubated his cultures for only twelve days compared to ninety days in Wildung and Schmidt's experiments.

Syers (5) reported that Golterman et al. (32) found that 8 to 44 percent of the NaOH- and H_2SO_4- extractable phosphorus in test sediments were available for uptake by the green alga Scenedesmus. Syers also reported that Sagher and Harris (33) found a substantial portion of sediment inorganic phosphorus could be utilized by algae. The "non-occluded fraction" of sediment phosphorus was found by the Wisconsin group to be the most available, while apatite phosphorus was found to have limited availability.

The ability of sediments to satisfy the phosphorus requirements of bacteria has been indicated by the work of Lee et al. (in Wisconsin paper). In cultures of Aerobacter aerogenes having sediment as the sole phosphorus source, they found that the adenosine triphosphate (ATP)

content of the bacteria was typical of normal cells.

Aside from being able to use phosphorus released from sediments by physical and chemical mechanisms, microorganisms seem to be able to mediate some of those mechanisms and compete with the inorganic phases of sediments for phosphorus. The role of bacterial metabolism in depleting oxygen from hypolimnetic water and sediments and from all but the top centimeter or so of littoral sediments is well recognized. The results, as examined by Mortimer (1, 2), can be dramatic increases in phosphorus concentration of lakes during turnover. Bacterial decomposition of organic matter results in the production of organic acids that can either increase sediment fixation of phosphorus by reducing pH (21, 24) or reduce or reverse fixation by chelation of aluminum and iron (24). Dead algae is a significant source of organic matter subject to bacterial decomposition. Jewell and McCarty (35) reported that a small portion of dead algae is rapidly biodegradable under aerobic conditions and that as much as 87 percent of dead algae takes longer than one year to decompose. Accumulated deposits of the refractory fraction might serve as a bacterial substrate for years after excessive algal growths would be controlled.

Pomeroy et al. (13) studied the uptake of P^{32} by estuarine sediments in both undisturbed and shaken sediments, with and without Formalin to poison microorganisms. The undisturbed sediment cores showed slow uptake of the added P^{32} with slight and not statistically significant differences between poisoned and non-poisoned sediments. Uptake was much more rapid in shaken sediments. Also, the amount of P^{32} taken up in the absence of Formalin was 50 to 100 percent greater

than when the sediments were poisoned, indicating a rapid biological uptake of phosphorus. Pomeroy deducted from his data that two rapid adsorption processes--one biological, the other physico-chemical--combine to yield an initial adsorption reaction that has a "half-time" of less than fifteen seconds. The initial reaction was followed by a more gradual reaction having a half-time of fifteen minutes which was not affected by Formalin and was, therefore, thought to be strictly physico-chemical. The investigators found that at equilibrium the orthophosphate content of water in contact with their estuarine sediments was 0.7 to 0.9 micromoles per liter (μM/1.) or 0.022 to 0.029 mg-P/1. They estimated the daily exchange across a submerged and undisturbed sediment surface to be on the order of 1 u mole of orthophosphate ($PO\text{-}3_4$) per m^2 or 0.031 mg-P per m^2. Stumm and Leckie (12) believe this to be in reasonable accord with their "maximum diffusional transfer rate" of 0.27 mg-P per m^2 per day.

Hayes and Phillips (18) reported a series of exchange experiments with P^{32} that they interpreted to show that bacteria are instrumental in determining the rate and direction of phosphorus exchange between sediment and water. The investigators initially determined that natural sediment cores obtained with a Jenkins sampler produced the same partition of P^{32} between sediment and water as artificial cores produced by centrifuging mixed sediments. For both types of cores, loss to the sediments of P^{32} added to the water was considerably more rapid and extensive in antibiotic-treated systems than in systems with active bacterial populations. This result was obtained in systems sparged with nitrogen as well as in systems aerated with oxygen. After a week, less

than 10 percent of the added P^{32} remained in the water of systems treated with antibiotic, while two-thirds remained in the untreated systems. Hayes and Phillips suggested that this result can be explained by either acceleration by bacteria of phosphorus release from sediments or adsorption of added phosphorus by the bacteria and conversion to organic phosphorus which would not participate in classical exchange reactions with sediment. Photosynthetic plants were excluded from these experiments by culturing the systems in darkness.

In other experiments with aquatic plants (Eriocaulon, Sphagnum, and Utricularia) without sediments, plants in antibiotic-treated cultures were able to effect a more rapid and extensive removal of added P^{32} from water than they could in the presence of bacteria. The authors concluded that the bacteria held phosphorus in the water and prevented a large loss to the plants. Conversion of the inorganic P^{32} to organic forms that are both held in the bacterial cell and excreted as soluble organic phosphorus prevents the plants from utilizing the P^{32}. Autoclaved phytoplankton was found to be unable to adsorb phosphorus. After sedimentation, P^{32} incorporation into sediments was accelerated by the dead algae. This uptake was attributed to increased bacterial activity on the sediment surface supported by the readily decomposable organic matter in the phytoplankton.

In another experiment, Hayes and Phillips analyzed the amount of P^{32} present in inorganic, soluble organic, and particulate organic forms over a period of ten days in sediment-water and water-only systems from which plants were excluded. Their findings were that bacteria in the sediments as well as an inorganic exchange mechanism of the sediments

compete with bacteria in the water for added phosphorus.

Hayes and Phillips concluded from their studies of non-sediment, bacteria, and higher plant P^{32} uptake that when "bacteria and higher plants compete for inorganic phosphorus, the bacteria get there first and change a part of it to organic forms which are apparently unavailable to plant use." Rhee (35) substantiated this competitive relationship with bacteria and algae. By determining the growth rates of a green alga, Scenedesmus sp., and a bacterium, Pseudomonas sp., together and separately in axenic culture, Rhee determined that: (1) the growth of the bacterium was more rapid than the alga; (2) the bacterial growth rate and the final cell volume at which the bacteria became P-depleted were unaffected by the presence of algae; (3) the reduction in algal growth rate in mixed culture coincided with P-depletion of the bacterium and occurred much sooner than in axenic culture; and (4) bacterial growth ceased when the phosphorus supply was depleted but algal growth continued to produce a final cell volume approximately four times as great as the cell volume at the point when external phosphorus was depleted. After analyzing the ability of algae to store phosphate, Rhee concluded that:

> Under non-limiting conditions, the rapid uptake of phosphate by bacteria (or their faster growth rate) may be sufficient for successful competition for it . . . For algae, on the other hand, phosphate is frequently a limiting factor in aquatic environments. With their slow growth rate, a mechanism for its storage would enhance their ability to compete for the element with bacteria.

The ability of some bacteria to liberate "fixed" inorganic sediment phosphorus is illustrated by the work of Harrison et al. (28). Sediments from Upper Klamath Lake were streaked on an undefined sediment extract and glucose agar to which sterile, finely powdered,

precipitated phosphate salts were added. The salts were $CaHPO_4$, $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$, $FePO_4$ or $Al_2(PO_4)_2$. Colonies of bacteria capable of dissolving the salts were recognized by cleared areas in the medium around colonies. Aerobic and facultative bacteria could dissolve the salts only under aerobic conditions. The mechanism responsible was chelation of the metals by organic acids excreted from certain bacteria.

The conclusion that may be drawn from the literature cited here is that the phosphorus equilibrium between sediment and water column in lakes is determined by a variety of both physico-chemical and biological processes. While the role played by biological processes is suspected by many investigators to be of considerable importance in affecting this equilibrium, relatively few studies have been published which attempt to quantify that role. None of the studies reviewed here consider long-term release of total phosphorus from aerobic sediments. The experiments reported here were designed to do this in the presence and absence of selected algae and bacteria.

MATERIALS AND METHODS

Introduction

The complexity of phosphorus interactions with lake sediments precluded controlled, *in situ* experiments that would specifically examine microbiotic participation in these interactions. In the present study, laboratory conditions were used that kept physical and chemical conditions constant and within the range of natural conditions. It was intended that the types of organisms present would be the primary independent variable in the experiment.

Lake sediments contained in small plastic dishes were submerged in a defined algal medium containing no phosphate. Medium was maintained in an aerobic condition by periodic aeration. These sediment cultures were inoculated with blue-green algae and/or bacteria. Cultures were harvested every five days for thirty-five days and subjected to a set of analyses selected to: (1) determine the changes in phosphorous distribution as a function of time between sediment, aqueous and biomass phases of the culture; and (2) follow the growth of algae and bacteria over time. Cultures poisoned with mercuric chloride served as a control to establish changes in phosphorus distribution in abiotic conditions. Details follow of the culture equipment and conditions, inoculum preparation, inhibitors used to control the types of biotic communities, culture separation, and the specific analyses conducted.

Figure 4 is a flow diagram of the procedures used in these experiments.

Growth Medium

Gorham's ASM-1 medium (36) was modified for use in these experiments. Phosphate salts were excluded. The potassium phosphate in Gorham's medium was replaced by potassium chloride to yield an equivalent concentration of potassium. EDTA was also excluded. As a chelating agent it would have effected a phosphate release from the sediments by chelating the iron in the sediment's ferric phosphate complexes. Sodium bicarbonate was added as a carbon source. Tris buffer ([hydroxymethyl]-amino methane) was included and the pH adjusted to 7.0 with HCl. All media were autoclaved at 15 psi and 121°C for 20 minutes. Table 1 presents the medium formulation used.

Growth Inhibitors

Four series of cultures were prepared. An abiotic series served to demonstrate the baseline of phosphorus transfer between sediment and water. Another series was inoculated with both blue-green algae and bacteria. A series with blue-green algae as the only intended inoculum and a series with bacteria as the only intended inoculum were included in an attempt to demonstrate each group's separate effect on phosphorus transfer. Reasons for selection of blue-green algae as the algae inoculum are given in Appendix B.

Neither the algae nor the bacteria inoculums were axenic. This, and the possibility that sediment sterilization (described in Appendix A) was not completely successful, necessitated the use of growth inhibitors. Table 2 shows the inhibitors used in each biotic

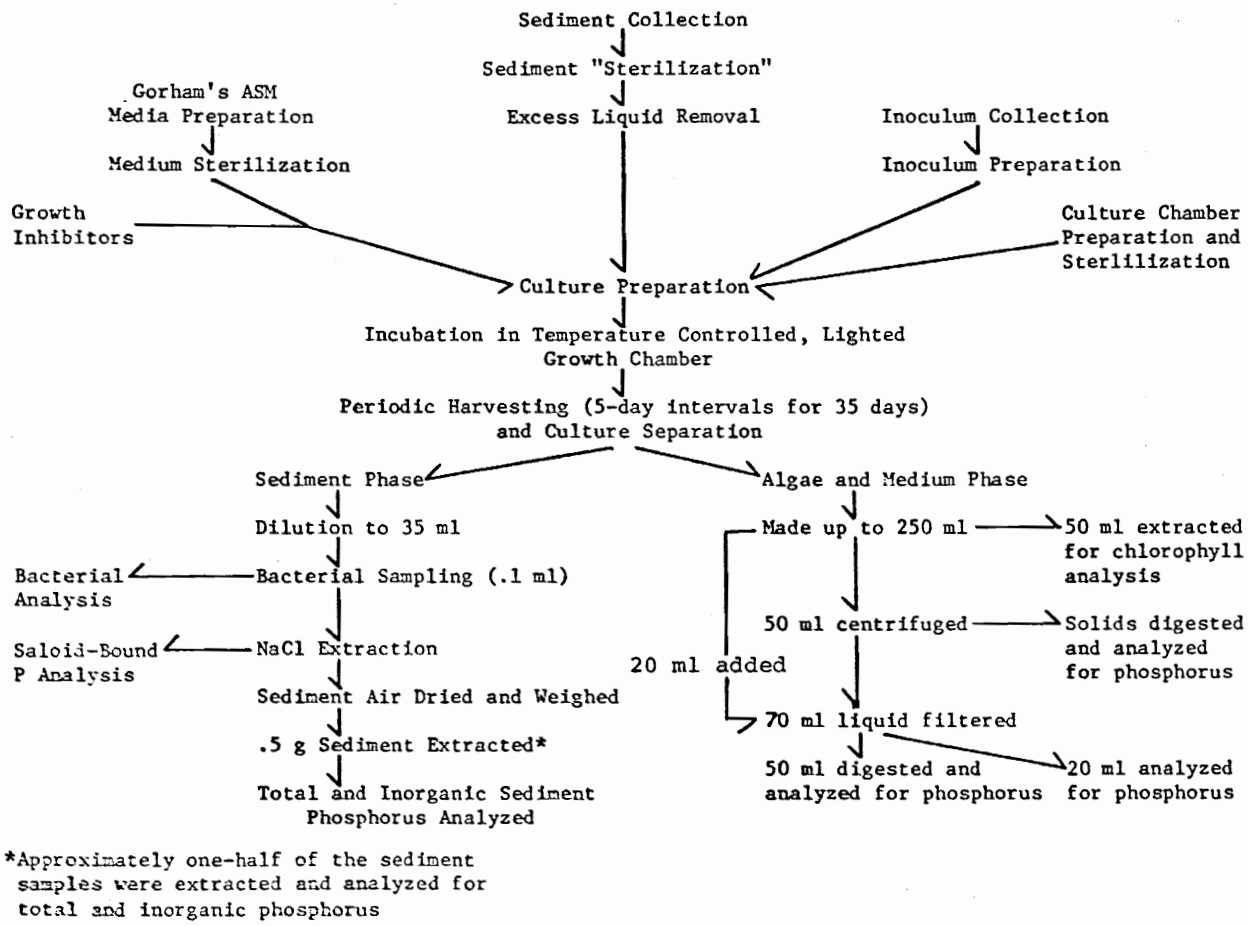

Sediment Collection
Sediment "Sterilization"
Excess Liquid Removal
Gorham's ASM
Media Preparation
Medium Sterilization
Inoculum Collection
Inoculum Preparation
Growth
Inhibitors
Culture Chamber
Preparation and
Sterlilization
Culture Preparation
Incubation in Temperature Controlled, Lighted
Growth Chamber
Periodic Harvesting (5-day intervals for 35 days)
and Culture Separation
Sediment Phase
Algae and Medium Phase
Dilution to 35 ml
Made up to 250 ml
50 ml extracted
for chlorophyll
analysis
Bacterial
Analysis
Bacterial Sampling (.1 ml)
50 ml centrifuged
Solids digested
and analyzed
for phosphorus
Saloid-Bound
P Analysis
NaCl Extraction
20 ml added
Sediment Air Dried and Weighed
70 ml liquid filtered
.5 g Sediment Extracted*
50 ml digested and
analyzed for phosphorus
20 ml analyzed
for phosphorus
Total and Inorganic Sediment
Phosphorus Analyzed
*Approximately one-half of the sediment
samples were extracted and analyzed for
total and inorganic phosphorus

FIGURE 4

TABLE 1

MEDIUM FORMULATION--MODIFIED GORHAM'S
(36) ASM-1 MEDIUM

The following chemicals were each dissolved in 100 ml of distilled, demineralized water. One ml of each solution was used in each liter of medium.

Chemical	Amount Per 100 ml	
$NaNO_3$	17.0	g
$MgSO_4 \cdot 7H_2O$	4.93	g(1)
$MgCl_2 \cdot 6H_2O$	4.06	g
$CaCl_2 \cdot 2H_2O$	2.94	g(2)
KCl	1.74	g(3)
$FeCl_3 \cdot 6H_2O$	.1081	g
H_3BO_3	.2474	g
$MnCl_2 \cdot 4H_2O$	.1385	g
$ZnCl_2$	.0436	g
$CaCl_2 \cdot 6H_2O$	.0019	g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.0129	g(4)
$NaHCO_3$	6.25	g

(1) equivalent to 2.4074 g of $MgSO_4$ used by Gorham

(2) equivalent to 2.2198 g of $CaCl_2$

(3) Potassium equivalent of 1.7418 g K_2HPO_4

(4) equivalent to .00001 g Mo^{++} per liter

TABLE 2

GROWTH INHIBITORS USED IN THE
FOUR BIOTIC SERIES

Series	Inhibitor(s)	Action
Algae plus Bacteria	Actidione 50 mg/l	Eucaryote poison (37)
Algae--only	Actidione 50 mg/l	Eucaryote poison
	Potassium Tellurite (K_2TeO_3) 100 mg/l	Bacteriostat (38, 39)
Bacteria--only	Actidione 50 mg/l	Eucaryote poison
	DCMU[I] 2.3 mg/l	Photosynthesis inhibitor (42, 43)
Abiotic	Mercuric chloride 40 mg/l	Preservative (41)

[I] 3 - (3:4 dichloro phenyl) - 1:1 dimethyl urea

series, their concentrations, and their inhibiting action.

Zehnder and Hughes (37) found that 50 mg/l of Actidione (cycloheximide) would kill most eucaryotes. However, even 200 mg/l failed to harm eleven species of blue-green algae.

Potasium tellurite (K_2TeO_3) is bacteriostatic in concentrations of 10 to 500 mg/l (38, 39). Bisalputra et al. (40), however, found that as much as one g/l failed to stop growth of two blue-green algae, Nostoc and Anabaena. The tellurite did concentrate in the algae's ribosomes, thereby turning the algae brown. Tellurite is not bacteriocidal, so the algae-only series cannot be considered to have lacked bacteria. It was expected only that the tellurite would inhibit bacterial reproduction.

The fate of tellurite in aqueous solution that is in contact with sediments was brought into question when freshly prepared tellurite-containing media gave an immediate reaction to the ascorbic acid method of phosphorus analysis (41). A light-blue, heavy precipitate formed when the fresh algae-only medium was analyzed. However, the aqueous phase of a five-day old culture containing the tellurite yielded none of this precipitate when analyzed for phosphorus. Also, neither sediment extracts not digests of algae grown in the tellurite-containing medium produced this precipitate. The precipitate was also formed when fresh tellurite-containing medium was analyzed by the stannous chloride method for phosphorus (41).

Kenyon (42) found that 10^{-5}M DCMU inhibits Photosystem II and CO_2 uptake in plants but does not inhibit heterotrophic growth. While some species of Oscillatoria can grow heterotrophically in the presence

DCMU, they require for this a supply of simple carbohydrate such as glucose.

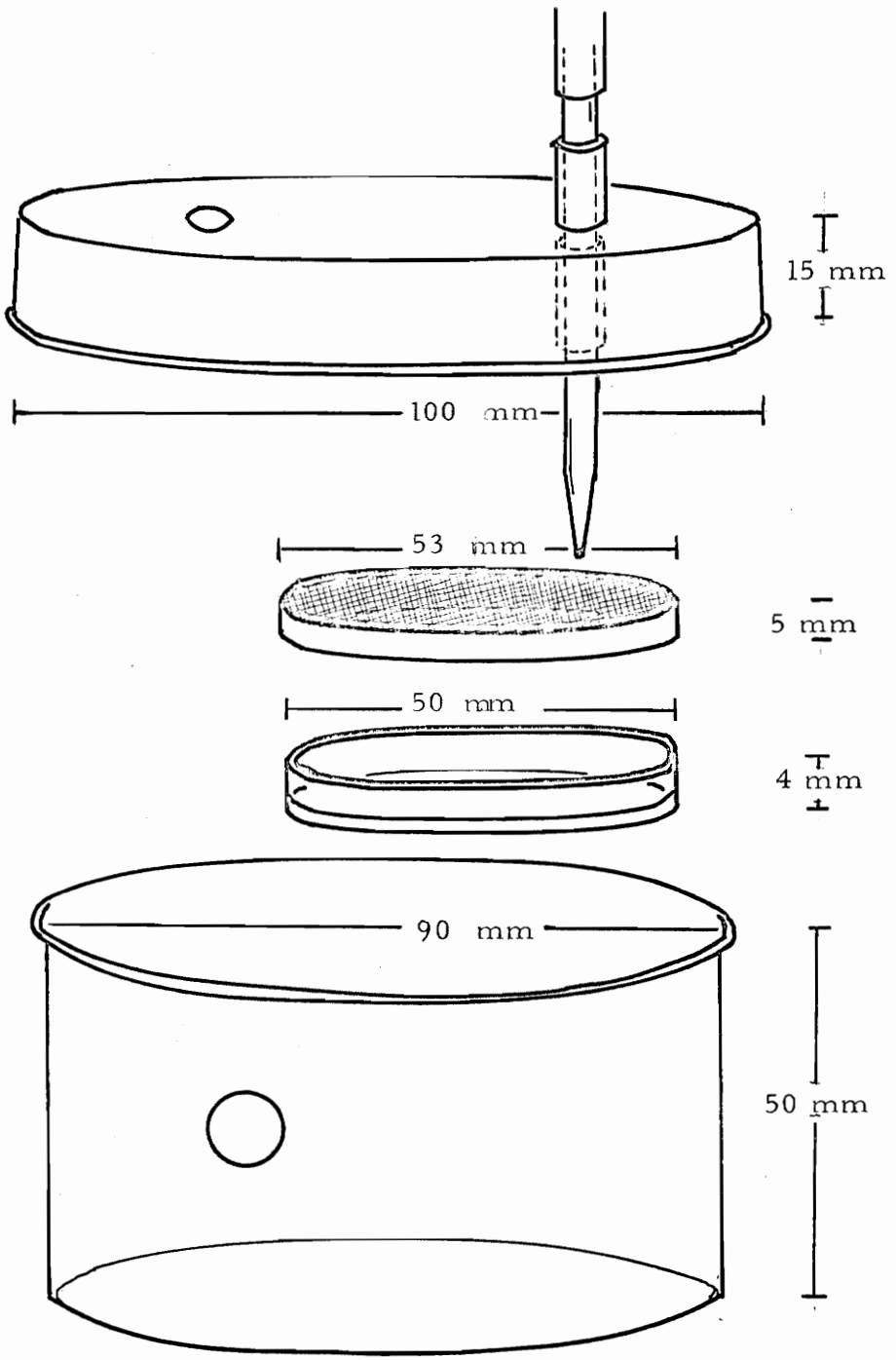
Culture Containers

One of the principal problems that had to be solved in the design of these experiments was the difficulty of separating algae from the sediment on which they grow. The problem was solved by containing the sediment in small dishes over which nylon mesh screens were placed. Algae were removed with the screen. This approach allowed destructive sampling of whole cultures having constant sediment surfaces and volumes but necessitated the use of multiple cultures for each sampling to obtain reliable estimates of the parameters sought. Three cultures for each of the four series were analyzed for each of the seven sampling periods.

A diagrammatic sketch of the entire culture containers is presented in Figure 5. The body of the containers were flat-bottomed, Pyrex crystallizing dishes (Corning 3140), 90 mm in diameter and 50 mm in height. Covers with aeration ports were made by drilling two one-quarter inch holes in 100 mm diameter glass Petri dish covers. One hole was plugged with cotton, and the other was fitted with an aeration tube. The aeration tube was made by cutting approximately two and a half inches of the tip end of a glass pipette and fixing it in position with two short lengths of plastic tubing fitted tightly around the pipettes above and below the Petri dish cover. Assembled culture containers were washed with diluted HCl, rinsed with distilled, demineralized water, and autoclaved.

The sediment dishes were the top halves of plastic Petri dishes

Figure 5 - Culture Container and Sediment Dish



that had an inside diameter of 50 mm, inside depth of 4 mm, and a volume of 7.85 cubic centimeters (Millipore Corporation: Bedford, Massachusetts). The ability of the plastic to sorb phosphates was checked by soaking several dishes in a 200 mg-P/l solution for four hours, rinsing them with distilled, demineralized water, soaking them in fresh, dilute HCl wash, and testing the HCl wash for phosphates after neutralizing to pH 4. No phosphorus was found, indicating that the plastic did not sorb phosphates. Sediment dishes were sterilized with a chlorine solution (3 mg/l) and rinsed with sterile water just before use.

Sediment dish screens were fabricated by gluing nylon fabric taken from ladies stockings onto rings cut from polyvinyl chloride pipe (2-1/8 inches in diameter). These screens fit snugly over the sediment dishes so that the nylon was in contact with the sediment surface. The screens were acid washed with dilute HCl and disinfected in the same manner as the sediment dishes. Non-toxicity of the materials used in the screen and sediment plate was indicated by growth of algae on all surfaces exposed to sunlight during the experiments.

Growth Conditions

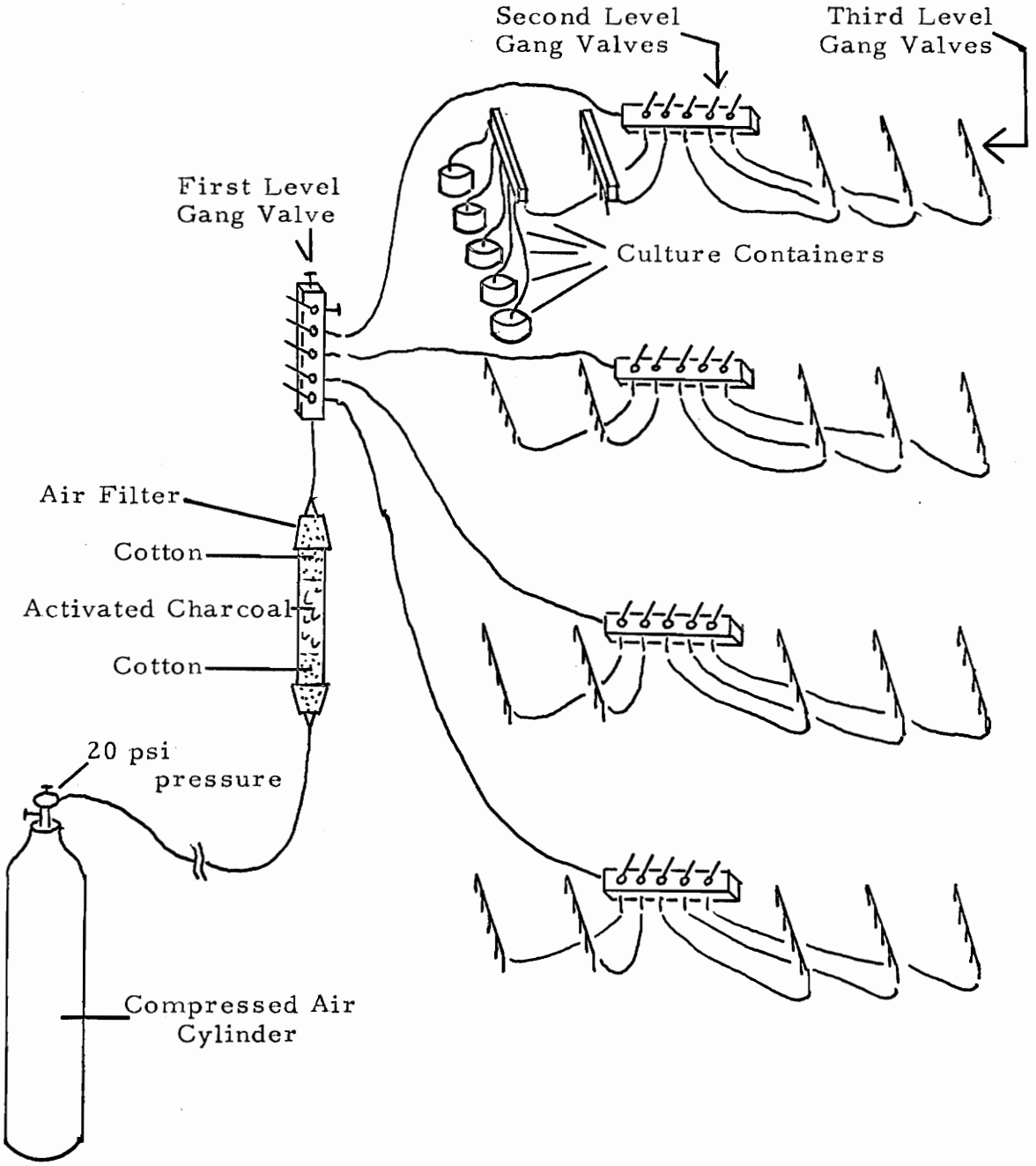
Periodic aeration of the medium placed in the culture containers during the experiments was required for two reasons: (1) to maintain all media near oxygen saturation in order to simulate actual littoral conditions by keeping the sediment surfaces and overlying water oxidized; and (2) to provide mixing, thus preventing phosphorus concentration gradients within the media. Deviations from oxygen saturation and completely mixed media otherwise would have required quantification.

Thirty minutes or more of aeration per day were provided to all microcosms simultaneously by the system illustrated in Figure 6. Compressed air was fed through rubber tubing to a filter cartridge containing two plugs of sterile cotton with granulated activated charcoal in between. The necessity to so treat the air supply was suggested by early experiments in which actively growing, mixed algal cultures gradually died off when continuously aerated with mechanically compressed air from air cocks in the lab, whereas similar non-aerated cultures thrived.

Filtered air was distributed to 100 outlets through Tygon tubing (3/16 inches in diameter) using three levels of five-gang, brass, aquarium valves. Initially, eighty-four microcosms were aerated. Air flow regulation was successfully accomplished by setting the air cylinder regulation at twenty pounds per square inch with all first- and second-level gang valves open and all third-level valves closed. Then, each third-level valve was carefully opened in turn until each allowed approximately one to three bubbles per second to pass through the media. After the initial setting, only minor adjustments to individual third-level valves were required during the course of the experiments. The ability of the aeration system to maintain high dissolved oxygen concentrations was evaluated by dissolved oxygen measurements presented in Part IV--Results.

Cultures were incubated at a constant 20° C in a lighted growth chamber. The fluorescent lights were automatically timed to provide sixteen hours of light and eight hours of dark per day. Aluminum foil was laid on each of four racks under the cultures to prevent increases in light intensities from the top rack to the bottom.

Figure 6 - Compressed Air Delivery Manifold. Detail minimized for clarity.



Sediment Collection and Preparation

Sediment was collected on June 11, 1974 from an area in the littoral zone of the Occoquan Reservoir, Virginia. Figure 7 locates the collection site. The locations of the major sewage treatment plants within the Occoquan watershed are also shown in Figure 7. These sewage treatment plants enrich the sediments of the Bull Run arm of the Occoquan Reservoir as noted by To (44). The collection site for the present thesis was originally a flood plain on the Fairfax County side of Bull Run. The site was flooded in 1957 with the construction of the Occoquan High Dam by the Fairfax County Water Authority. When the Occoquan Reservoir is at its pool level of 120 feet above mean sea level, the collection site is covered with two to four feet of water. At the time of collection, the reservoir was near pool level. The water was turbid as a result of previous rainfall.

Approximately 1.4 liters of flocculent surface sediment was collected by:

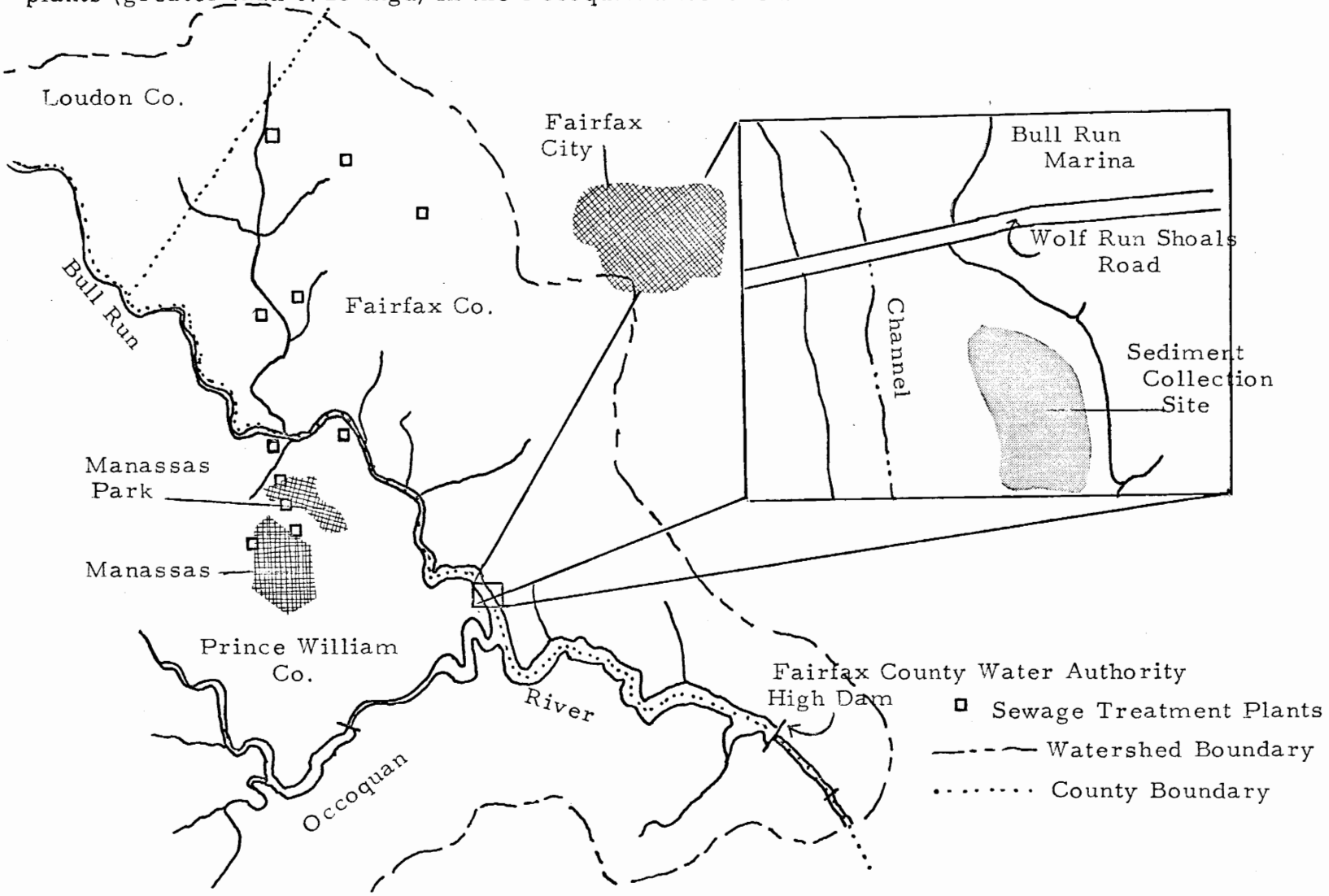
1. dredging numerous plugs of sediment from the bottom using a brass Eckman dredge (six inches by six inches);

2. carefully decanting water off the sediments before removing the dredge from the reservoir. This step was necessary to present disturbance of the sediment horizons;

3. emptying the two to three inch deep sediment plugs into a flat-bottomed pan and carefully removing the dredge;

4. scraping approximately one centimeter of flocculent sediment off the surface of the sediment plug with the long side of a seven

Figure 7 - Location map for the sediment collection site and the major sewage treatment plants (greater than 0.25 mgd) in the Occoquan watershed.



inch long stainless steel lab-scoop;

5. passing the collected sediment through a No. 20 brass-wire sieve (.0331 inch diameter openings). Very little of the surface sediments failed to pass through this sieve, plant, stalks, and twigs being the primary materials retained;

6. pouring sieved, surface sediment into polyethylene bags having water-tight closures. Filled bags were stored in ice until refrigeration was possible. Sediments were kept under refrigeration until they were treated with ethylene oxide on July 1-6, 1974.

Preliminary experiments on sediments collected earlier in the year demonstrated that zooplankton, especially crustaceans and tubifex worms native to these sediments were very active in disturbing sediments kept in glass culture containers. As the experimental design required that sediments remain in their dishes and that the algae and bacteria cultures selected for inoculation not be subject to predation or competition, a means of eliminating or reducing the biotic populations in the sediments was sought. Autoclave sterilization was ruled out as it would radically alter the forms in which the phosphorus occurred in the sediments. Cold sterilization with gaseous ethylene oxide was chosen.

The sterilization process is described in Appendix A. The sterilization was not completely successful but it did greatly reduce the number of bacteria in the sediments.

The ethylene oxide treated sediments compacted to approximately 80 percent of their initial volume, thereby releasing water as a supernate. Although this supernatant water: (1) would have served as a source of available phosphorus if mixed back into the sediment; and (2) repre-

sented a significant component of the natural sediments, it was disregarded. Otherwise, recompaction of the sediments in the sediment dishes would have disrupted the intended sediment-screen-water interface.

Inoculum Collection and Preparation

The algal and bacterial inocula were prepared from populations obtained at the site of sediment collection. No attempt was made to isolate or identify species prior to their use in these experiments.

Bacteria were grown in Pond Water Medium made of equal parts of sterilized, distilled, demineralized water and sterilized water from the Occoquan Reservoir taken at the site of sediment collection. Yeast extract and peptone were added at a concentration of one percent and the medium was sterilized again. A small amount of fresh surface sediment was added to this medium at the time that sediments were collected. The culture was incubated in the lighted experimental growth chamber at 20° C. Transfers were made every three days to fresh Pond Water Media and the culture flasks were shaken several times a day to keep the culture aerobic.

The source of the algae inoculum was a mixed culture taken from a preliminary study to determine the nature of community algae growth in artificial media. Occoquan sediments--taken in March, 1974 from the previously described sampling point--had been incubated in an inorganic, liquid nutrient medium which lacked phosphorus. Algae in the sediments thrived and produced a luxurious growth on the sediment surface and in the media. Filamentous and colonial blue-green algae dominated the culture.

In order to eliminate algae other than the blue-green algae and to eliminate eucaryotes which might consume the blue-green algae in the experiments, these mixed algae cultures were transferred without sediments to a culture medium containing Actidione (cycloheximide). To promote growth of the blue-green algae, Gorham's ASM-1 medium was fortified with sterilized soil extract made from Occoquan sediment (45). Approximately 20 percent of the final inoculum medium was soil extract. The Actidione concentration was approximately 75 mg/l. This blue-green algae culture was incubated at 20° C in a lighted growth chamber for fourteen days prior to inoculation into the experimental cultures.

Culture Preparation

Sediments previously treated with ethylene oxide (see Appendix A) were stirred with a magnetic stirrer and stirring bars prior to placing them in the plastic sediment dishes. Sediment was transferred to the dishes using plastic pipettes with their tips removed. Sediment was leveled with the top of the dishes by scraping the excess away with a metal straight edge. No attempt was made to weigh the sediments in each dish at this time. Dry weights of the sediments were determined after culture incubation and separation.

Sediment dishes were covered with disinfected nylon screens and then placed carefully in the culture containers. Modified Gorham's ASM-1 medium was added to the culture containers by siphon. Approximately 175 ml of medium was added to each container. Some sediment adhering to the nylon screens was released from the sediment surface when the medium first covered the screens. This loss of sediment to

the aqueous phase was small compared to the total sediment volume, but might have been of some consequence by increasing the total sediment surface exposed to the aqueous phase.

Aliquots of the bacteria and/or algae inocula (0.5 ml) were pipetted into the prepared culture containers. The algae inoculum had to be blended briefly in a Waring blender prior to inoculation since the algae were growing in clumps. Cultures were covered with the glass lids, placed in a lighted, temperature-controlled growth chamber, and fitted to the pre-set air manifold outlets.

Culture Separation

Beginning five days after inoculation, three cultures from each of the four series were removed from the growth chamber every five days for thirty-five days.

Each culture was initially separated into a sediment phase and a combined medium-biomass phase. The sediment and sediment dish with nylon screen in place were carefully lifted out of the culture with acid-washed, metal tongs. The screen was gently lifted off and the sediment adhering to it and/or the algae mat was washed into pint Mason jars with a spray of demineralized, distilled water. The spray, delivered from an acid-washed Windex bottle, successfully separated the sediment from the algae mats because the algal mats adhered to the screen more strongly than the sediment adhered to either the mat or screen. Algae adhering to the outside of the plastic dishes were scraped back into the medium-biomass phase of the culture. The sediment and dish were drooped into the Mason jar and the total volume of sediment, water, and dish made up to approximately 50 ml with distilled, demineralized

water. Bacteria counts, sediment dry weight, and saloid-bound phosphorus were determined from these sediment mixtures as described later. Total and inorganic phosphorus were determined on about one-half of the culture sediments.

The inside surfaces of the culture containers were scraped free of adhering biological growth and the medium-biomass phases were poured into beakers calibrated at 250 ml. Algae from the nylon screen was added to this phase and the volume was brought up to the 250 ml mark. During the first four samplings, algae was removed from the screens by three to ten seconds of sonic oscillation in 25 ml of water. The oscillator broke down prior to the fifth sampling. Algae removal from the screen for the fifth through seventh samplings was accomplished by rigorous scraping of the screen with a plastic policeman.

The medium-biomass phases were blended briefly prior to subsampling in a glass Waring blender to disperse algae clumps.

Chlorophyll Analysis

The nature of information on algae growth that was required for these experiments did not necessitate cell counts or other absolute measures of algal biomass. A relative index of the amount of algae in cultures was based upon the height of the chlorophyll-a absorption peak at 663 mμ. A chlorophyll extraction method described in the U.S. Geologicial Survey publication, Collection and Analyses of Aquatic Biological and Microbiological Samples (46), was used.

Fifty ml of the blended medium-biomass mixture was filtered through glass-fiber filters (Whatman Grade GF/A). The filters were macerated by mortar and pestle with some spectrophotographic grade 90

percent acetone, transferred to a glass, pestle-type tissue grinder, and homogenized. The acetone-filter slurry was transferred to polyethylene centrifuge tubes and made up to 15 ml with the 90 percent acetone. After centrifuging for ten minutes, the supernatant was spectrophotometrically scanned in a Model Perkin-Elmer scanning spectrophotometer using a one Å light path width. The difference between the 750 millimicron (mu) and the 663 mu optical densities was used without further calculation as a linear index of the amount of chlorophyll extracted. Values are reported as optical density per 50 ml extracted (o.d./50 ml).

Analyses which correlate algal biomass concentration to the optical density of the chlorophyll-a peaks for the blue-green algae culture are reported in Index B.

Bacteria Analysis

Bacteria counts in the sediment-water mixtures were made by serially diluting a small amount of the mixture and plating the dilutions on solid nutrient agar.

One-tenth ml of the approximately 50 ml of the sediment-water mixtures was taken by serological pipette and introduced into 9.9 ml of demineralized, distilled water in 15-ml glass dilution tubes that had been sterilized by autoclave. From two to four serial dilutions using these same volumes (100:1 dilution) were made of the initial dilution. The number of dilutions made depended upon the expected number of bacteria. One-tenth ml of each of the dilutions were transferred, after shaking, to Petri plates containing sterile Pond Water Agar. These plates were made using a 1.5 percent agar solution of the Pond Water Media, described earlier, in which the original Occoquan bacteria

had been collected and incubated. The dilutions were spread evenly over this Pond Water Agar with a flame-sterilized, glass rod. Inoculated plates were incubated for three days in the 20° C light chamber that was also used for incubuting the experimental cultures. Then the number of colonies per plate were recorded. Reported bacteria counts are averages from three similar plates for the dilution which yielded the highest, countable number of colonies.

Phosphorus Analysis--Medium-Biomass Phase

The distribution of phosphorus in the medium-biomass phases was determined by: (1) centrifugally separating the suspended solids from the liquid; (2) testing for total phosphorus in the solids ("total centrifugable solids phosphorus"); and (3) testing for total phosphorus in the liquid ("total soluble phosphorus"); and (4) testing for ortho-phosphate in the liquid ("Medium Orthophosphorus").

Total Centrifugal Solids Phosphorus

Fifty ml of the blended medium-biomass phases was centrifuged for ten minutes. The supernatant was saved for further analysis. The algae, bacteria and/or sediment that centrifuged out was transferred to acid-washed microkjeldahl flasks. The centrifuged material was then digested by a sulfuric acid-nitric acid method recommended by Lee et al. (47) that they reported to have yielded results on algae comparable to a more rigorous and hazardous perchloric acid digestion. The digest was neutralized to a pH of approximately 2.8 with 1:1 ammonium hydroxide (NH_4OH) using 2, 4 dinitrophenol indicator. This was brought up to the

original concentration of 50 ml with distilled, demineralized water. Twenty ml of this solution was tested for phosphates by the ascorbic acid method (41). In instances where dilutions were necessary, 5.0 or 10.0 ml were made up to 20 ml for analysis.

Total Soluble Phosphorus

Supernatant from the previously described centrifugal separation and an additional 30 ml of the medium-biomass phase were vacuum filtered through glass-fiber filters (Whatman Grade GF/A). Fifty ml of the filtrate was digested in the same manner as the centrifuged solids. Analysis of this digest yielded total soluble phosphorus. This fraction when added to the total centrifugal solids phosphate results in an estimate of the total phosphorus in the medium-biomass phase. A small negative error in the total phosphorus in this phase was due to occasionally incomplete centrifugation of solids.

Medium Orthophosphorus

Twenty ml of the undigested filtrate was analyzed for phosphorus by the ascorbic acid method. This fraction is generally considered to represent the form of phosphorus that is available for algal assimilation.

Phosphorus Analysis--Sediment

Three phosphorus fractions were analyzed in the culture sediments: (1) saloid-bound; (2) inorganic; and (3) total. The inorganic and total phosphorus analyses were made on sediments after saloid-bound phosphorus was extracted.

Saloid-Bound Sediment Phosphorus

This fraction includes interstitial and loosely bound inorganic phosphorus. It is the most chemically mobile phosphorus in the sediments and is likely the most available for biological utilization (27).

The method for analyzing the saloid-bound fraction was adopted from the sediment phosphorus fractionation method of Chang and Jackson (48). A sufficient amount of 3N NaCl (usually about 10 ml) was added to the sediment-water mixture in Mason jars to produce an equivalent .5N NaCl concentration. The Mason jars were shaken for four hours on an oscillating shaker. The shaken mixture was transferred to acid-washed, polyethylene centrifuge tubes and centrifuged for ten minutes. The supernatant was poured off, made up to 100 ml, and tested for inorganic phosphorus by the ascorbic acid method.

Inorganic and Total Sediment Phosphorus

The sediments were then transferred to acid-washed, air-dried, tared, porcelain, evaporating dishes and left to air dry at room temperature. Elevated evaporating temperatures were not used in order to avoid altering the phosphorus fractions.

The dried sediment-evaporating dish weight was determined in order to calculate sediment weight. The sediment was then pulverized in the dishes using a porcelain pestle and stored in sealable polyethylene bags.

Total and inorganic phosphorus remaining in approximately one-half of the sediments were determined by an extraction process after Mehta et al. (49). Sommers et al. (50) found the Mehta method to be superior to four other methods for determining organic and inorganic

sediment phosphorus. One-half gram of the air-dried sediment was extracted serially by concentrated hydrochloric acid, 0.5N NaOH at room temperature, and 0.5 N NaOH at 90° C. The extracts were combined and made up to 250 ml with distilled, demineralized water.

To determine total sediment phosphorus, 20 ml of the combined extract was digested in microkjeldahl apparatus using sulfuric and nitric acid followed by perchloric acid (47). The digest was transferred to a graduated cylinder and made back up to 20 ml. One ml of this solution was diluted to 20 ml and tested for phosphorus.

One ml of the undigested extract was made up to 20 ml and tested for phosphorus to determine inorganic sediment phosphorus.

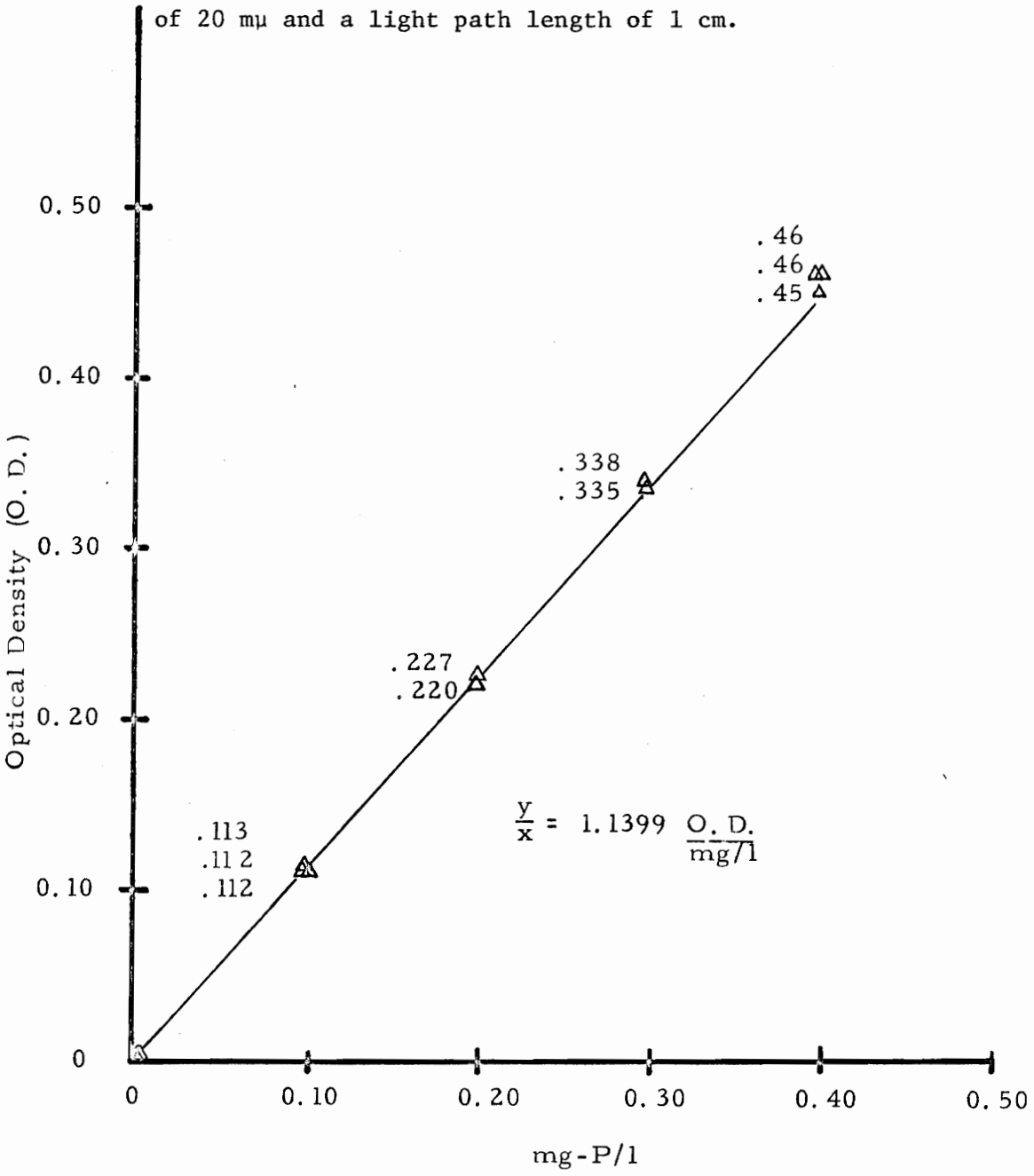
While Mehta et al. had used a stannous chloride colorimetric determination for phosphorus, the ascorbic acid method was shown by the method of standard additions to be free of interferences from the extract. In addition, Mehta's perchloric acid digestion was amended to include a prior sulfuric-nitric acid digestion to reduce the possibility of accidents.

Standard Curves and Estimates of Error

Phosphorus Analysis

All analyses of phosphorus were performed by the ascorbic acid method (41). The standard curve generated by analysis with a Bausch and Lomb Spectronic 20 colorimeter is presented in Figure 8. Regression analysis of the data showed that the slope of the line was 1.1399 optical density per mg/l (o.d./mg/l). The standard error of the esti-

Figure 8 - Standard curve for orthophosphate in water. Phosphorus reported as P. Curve generated on a Bausch and Lomb Spectronic 20 set at 880 mμ with a spectral slit width of 20 mμ and a light path length of 1 cm.



mate was ± 0.0047 o.d./mg/l.

Of the various phosphorus analyses conducted for this study, the one thought likely to produce the most severe interferences were the inorganic and total phosphorus analyses of the Mehta sediment extracts. The extracts were all highly colored and may have contained extracted sediment fractions that could have interfered. The method of standard additions was used to test for interference. Two ml of the digested or undigested extract plus known quantities of inorganic phosphate in a standard solution were diluted to 20 ml and tested for orthophosphate. Interferences from two digestion techniques were examined by the method of standard additions: a sulfuric acid-nitric acid digestion (41) and the same method followed by digestion with 70 percent perchloric acid (47). The slopes of the various curves obtained and their standard error of the estimate are presented in Table 3. The low value for the slope of total phosphorus digested without perchloric acid was thought to be due to a greenish color left in the digest. This color, and the change in slope associated with it, were not found in digests which received the additional treatment with perchloric acid. For this reason, the perchloric acid digestion was used in the analysis of total sediment phosphorus.

Lee et al. (47) found that equivalent results were obtained on samples of algae with the sulfuric acid-nitric acid digestion and the same digestion followed by perchloric acid digestion. Therefore, the perchloric acid digestion was not used in the analysis of total soluble phosphorus in the media or total phosphorus in media contrifugable solids. Replicate analyses of samples for these two parameters were not performed.

TABLE 3

SLOPES AND STANDARD ERROR OF THE ESTIMATES
FOR FOUR PHOSPHORUS ANALYSES

Analysis	Slope o.d./mg/l	Standard Error of the Estimates
Orthophosphate in distilled, demineralized water	1.1399	± 0.0047
Inorganic Phosphorus in Mehta Extract	1.1085	± 0.0099
Total Phosphorus in Mehta Extract-- Sulfuric acid-nitric acid digestion	1.0125	± 0.0091
Total Phosphorus in Mehta Extract-- Perchloric acid digestion	1.1100	± 0.0199

An estimate of error, therefore, was not possible.

In order to determine the inorganic and total phosphorus content of the original sediments, a number of sediment samples were air dried, extracted by the Mehta procedure, and analyzed. Three sediment samples were analyzed in duplicate by the Mehta procedure without prior extraction by a weak salt solution. The results of these analyses are summarized below:

	Mean (mg/.5g)	Standard Deviation (mg/.5g)
Sediment Inorganic--P	0.48214	0.00755
Sediment Total--P	0.78199	0.03682

Preliminary estimates of error in the weak salt solution extraction of sediments using NH_4Cl (48) showed that all NH_4Cl available was contaminated with phosphorus. The cleanest .5N NH_4Cl solution made had a concentration of approximately 1 mg-P/1. Therefore, a solution of NaCl which showed no phosphorus concentration was used to extract saloid-bound sediment phosphorus in this study.

Chlorophyll Analysis

During the course of the study, few algae-containing samples were extracted and analyzed for chlorophyll in replicate. The only culture sample that was analyzed more than once was a fourth-sampling-period algae-plus-bacteria culture. Results of the three analyses were .30, .31, and .35 o.d./50 ml. The standard error of the mean for these values is $\pm$.021 o.d./50 ml which is approximately four times as large as the spectrometer reading error, .005 o.d., and approximately 7 percent of the mean.

Other chlorophyll replicates were performed in conjunction with the non-sediment algal growth study reported in Appendix B. The standard error of chlorophyll readings in these experiments increased with increasing values of the readings to yield an apparently constant standard error as a percent of the mean that was between 6.5 and 10.1 percent.

It was determined from this finding and from review of the techniques used that the main source of error was inexact measurement of the chlorophyll extract volume. This error was thought to be non-directional.

Bacteria Counts

A small source of error in the bacteria counts was recognized after the fourth sampling date. It had been wrongfully assumed during the design of the analysis that the distilled, demineralized water used to wash sediments from the nylon screens and algae mats was relatively bacteria-free. Plating and culturing of a small amount (less than 1 ml) of this wash water on the Pond Water Agar revealed that it contained approximately 100-500 bacteria per ml that could thrive on the agar. Subsequently, all culture separations were performed with autoclaved, distilled, demineralized water. The error caused by this oversight was thought to be small since 0.1 ml of the sediment-water mixture was diluted by at least a factor of 100 prior to plating. The error was not likely to have been more than 1×10^3 bacteria per ml of mixture. By comparison, the lowest bacterial count from the experiments was 37×10^3 per ml.

The two greatest sources of error in estimating the bacterial populations of the sediment were: (1) the unquantified volume of the sediment-water mixture; and (2) the small volumes (0.1 ml) of the mixture for starting the dilutions. Sediment-water mixture volumes generally were about 35-50 ml, but some may have been as little as 30 ml or as great as 75 ml. In comparing bacterial counts, these differences in dilution may contribute a 50 percent error. Since only 0.1 ml of the mixture was utilized (to prevent significant change in the sediment weight), pipetting errors would have increased the overall error in the analysis. A maximum cumulative error of $\pm$ 100 percent was estimated for bacterial counts. However, this degree of error would do little to change the order of magnitude of values to be discussed in the section regarding interpretation of results.

The errors involved in weighing sediments were not quantifiable. The balance used to weigh evaporating dishes and sediments was accurate to $\pm$ 0.1 mg. This error is thought to be negligible in comparison to loss of sediments during prior analyses and the variations caused by not being able to oven-dry either sediments or evaporating dishes. Oven-drying would have changed the distribution of phosphorus in the sediments. A net negative error of perhaps one to two percent was estimated for sediment loss. Errors due to differences in water content were expected to be random and on the order of $\pm$ 0.1 gram per sample.

RESULTS

Data acquired by methods described in the previous section are tabulated in Appendix C. Significant aspects of the several parameters are discussed below after review of phosphorus flux between sediment and medium in the four biotic series.

Phosphorus Flux

The changes over time in total phosphorus present in the liquid phases of the four biotic series are shown in Figure 9. Points on each line are averages of total liquid phase phosphorus for three cultures. The total liquid phase phosphorus is the sum of total centrifugal solids phosphorus and total dissolved phosphorus.

It is obvious that the two series that contained algae realized more rapid and more extensive shifts of phosphorus into the liquid phase from the sediments than did series in which algae was excluded. In both the abiotic and bacteria-only series, approximately one-half of the phosphorus transfer occurred in the first five days of incubation with gradual increases thereafter.

Although sediment dry weight and, therefore, sediment total phosphorus varied from culture to culture, a constant mass of phosphorus in each culture has been assumed in order to illustrate in Figure 10 the relative percent of culture phosphorus transferred to the medium-biomass phase in each series. The mass of phosphorus in the cultures was calculated using the average sediment dry weight, 4.113 g, and the total

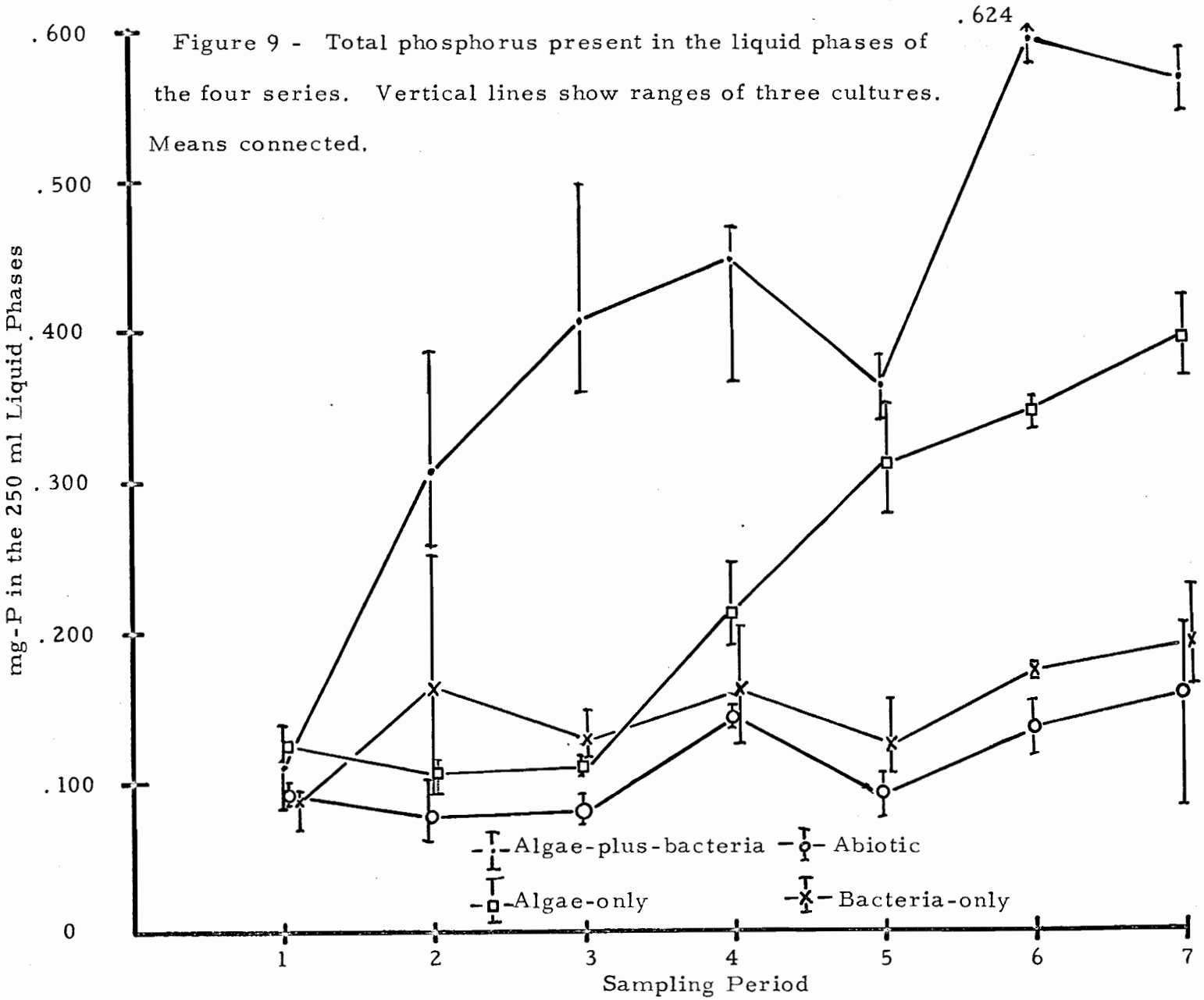
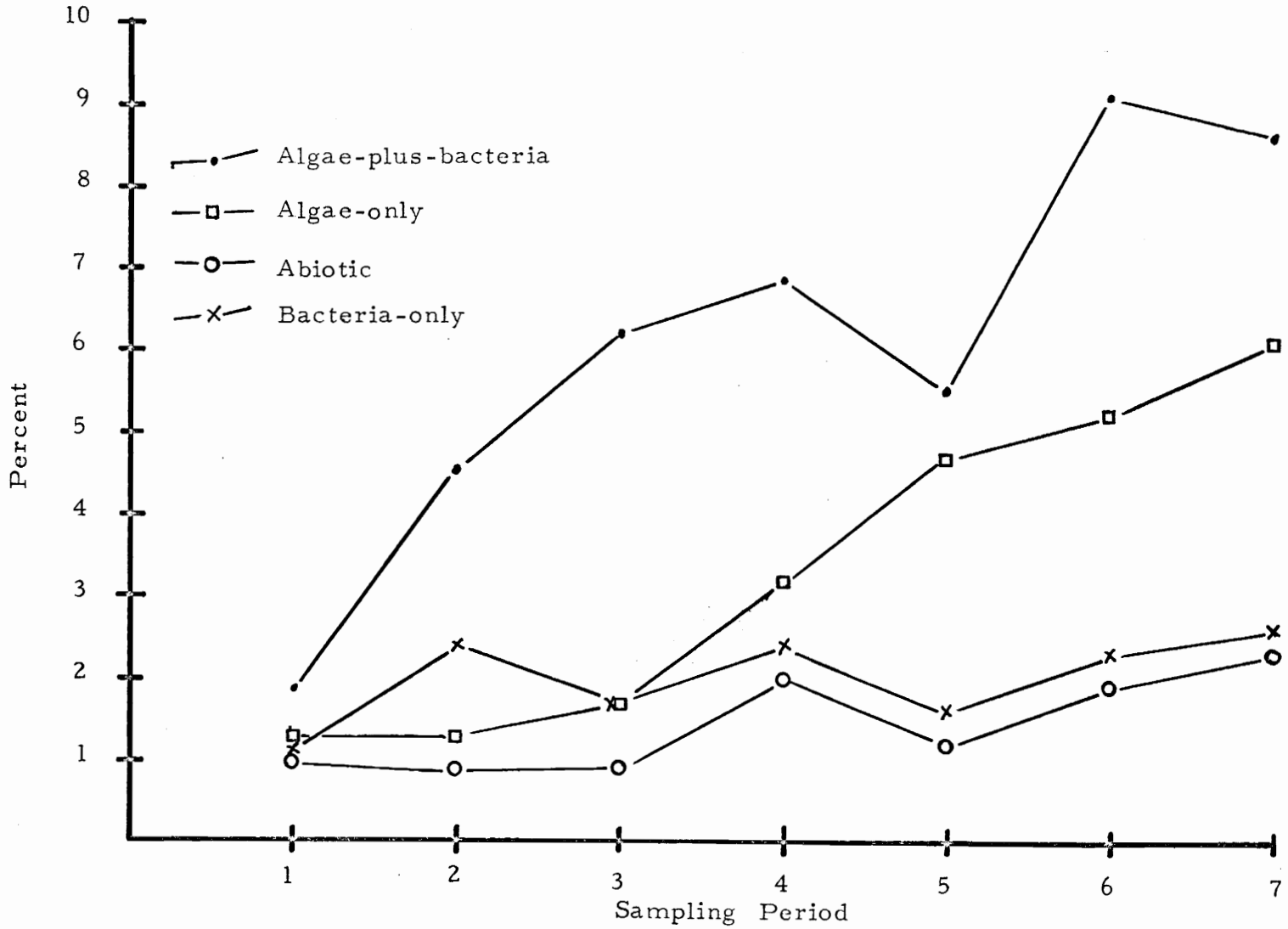


Figure 9 - Total phosphorus present in the liquid phases of the four series. Vertical lines show ranges of three cultures. Means connected.

Figure 10 - Percent of Calculated Total Culture Phosphorus Present in the Media-biomass Phase of Each Series.



sediment phosphorus concentration of the original sediment, 1.564 mg-P/g.

Algal Growth

Growth of algae was visible in the algae-plus-bacteria cultures three days after inoculation. By five days, the time of the first sampling, algae growth was sparse but evenly distributed over the sediment surfaces. The algae at that time were attached primarily to the sediments, not the nylon screen, and separation of algae and sediments was not complete. By the tenth day, the time of the second sampling, a cohesive algal mat had grown between the sediment surface and the nylon screen. Portions of the mat adhered to the screen and the rest remained attached to the sediment surface. The mat attached to the sediment surfaces was easily lifted off and washed free of sediment. By the fourth sampling period, twenty days after inoculation, the mat was quite cohesive and well attached to the nylon screen. Sediment attached to the algae mat was easily washed off without tearing the mat. By the last sampling date, a considerable amount of algae had grown on the walls of the culture containers.

Algal growth in the algae-only series was not so rapid. The mats found were never as cohesive as the mats in the algae-plus-bacteria series. Algae tended to grow in clumps and were light to dark brown in color whereas the mats in the algae-plus-bacteria cultures were distinctly green. The brown color of the algae was attributed to the uptake of tellurite as described by Bisalputra (40). The algae adhered poorly to the sediments but were well attached to the nylon screens. The algae clumps were somewhat mucilaginous and were at times difficult to remove from the nylon screens. As in the algae-plus-bacteria cultures, the

proportion of algae growing on the walls of the culture container increased with time.

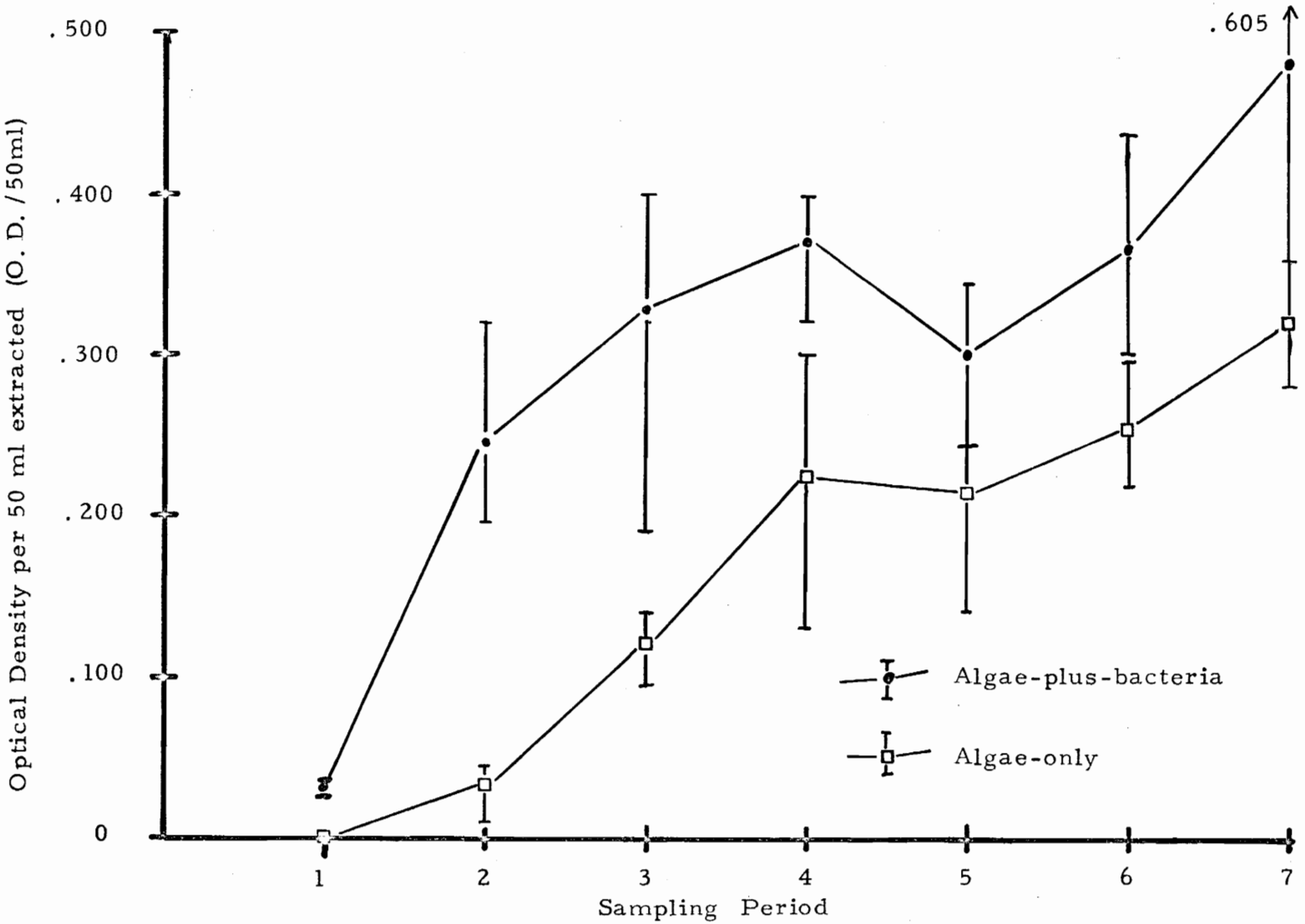
Approximately 2.5 percent of algae was suspended in the culture medium at the end of the experiment. Twenty-five ml aliquots of undisturbed medium from seventh-run algae-only and algae-plus-bacteria cultures were filtered, extracted, and analyzed for chlorophyll. The chlorophyll reading for each sample was .01 o.d./50 ml (optical density can be multiplied linearly) compared to an average of .40 o.d./50 ml for all of the algae in other cultures of the same age (thirty-five days). The remainder of the algae was growing on sediment and container surfaces.

Generation of gas, presumably oxygen, under the screens was noted in cultures of both algae series. By twenty-two days after inoculation, one of the algae-plus-bacteria cultures had enough gas produced to raise the nylon screen away from the sediment surface. By the end of the culture period at thirty-five days, all nylon screens were raised in the remaining cultures containing algae.

Figure 11 shows the absorbance of the chlorophyll extracts for both algal series plotted against length of incubation in days. Ranges are shown to illustrate the variability of the results between similar cultures. Averages for each set of three cultures are connected by lines to demonstrate growth trends for the two algal series.

Appendix B includes description and discussion of experiments with non-sediment algal cultures intended to serve as controls for the sediment cultures. A description of the organisms found in an algae-plus-bacteria culture and an algae-only culture is also given in Appendix B.

Figure 11 - Optical Densities of Chlorophyll Extracts. Vertical lines demonstrate ranges of three cultures. . Sampling period means are connected.

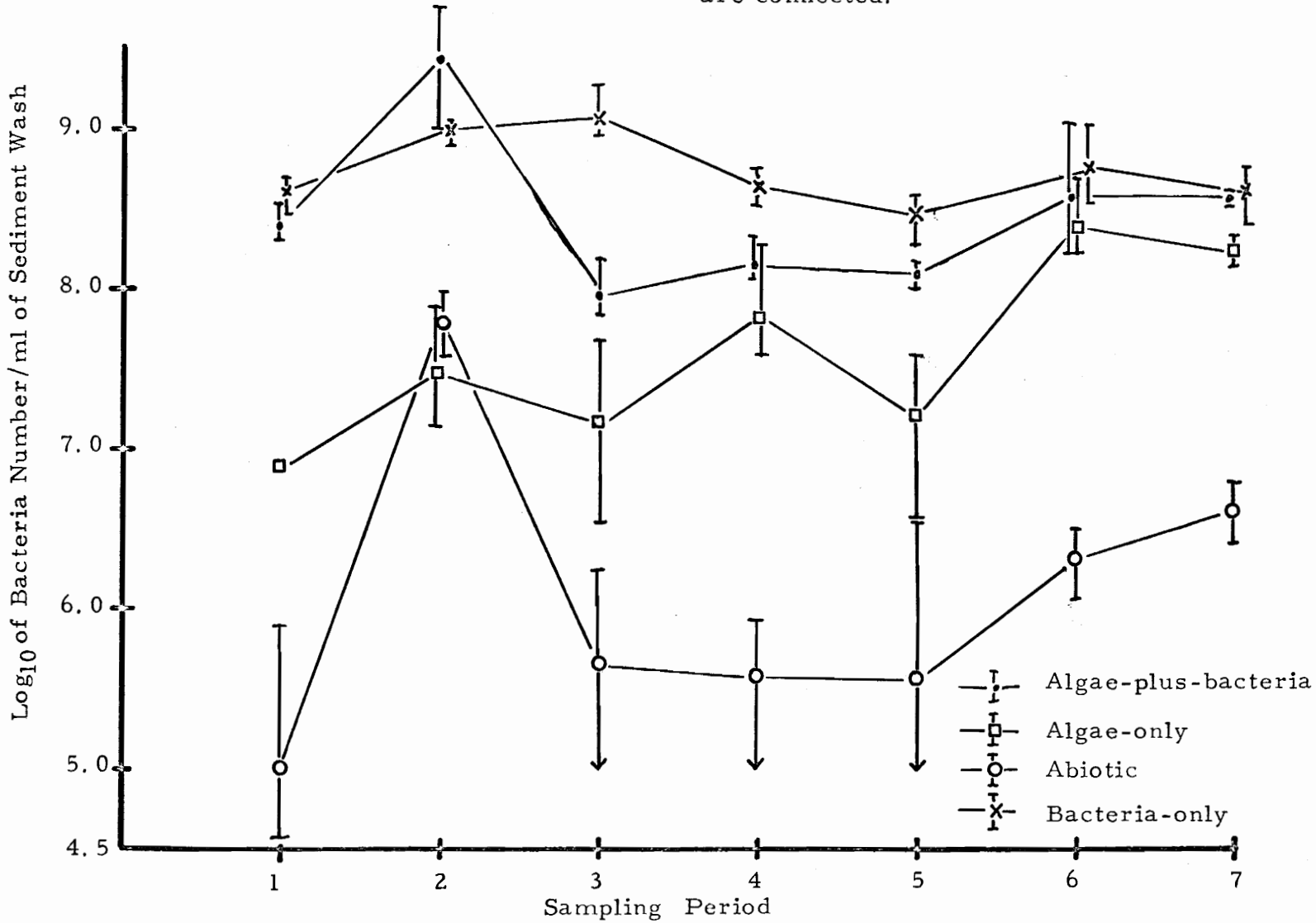


Bacteria Growth

Figure 12 displays the changes in sediment-water mixture bacteria counts. Lines in Figure 12 connect the means of $\log_{10}$ values for each biotic series. With the exception of the second sampling period, the abiotic series had the lowest, bacteria-only had the highest, and algae-containing cultures had intermediate sediment-water mixture bacteria counts. The bacteria counts in the supposedly abiotic sediments indicate that the mercuric chloride inhibitor was not completely effective within the sediment mass. Similarly, the algae-only series, while its sediment bacteria counts were reduced, were found by this analysis to contain substantial bacterial populations. Bacterial growth at the sediment-water interface and in the medium cannot be estimated from this data. Greater differences in the sediment bacteria counts may have been obtained if the bacterial inhibitors, mercuric chloride, and potassium tellurite had been mixed with sediments prior to culture preparation instead of simply being added to the medium.

In preliminary experiments, non-aerated cultures made with untreated sediment demonstrated heavy discoloration of sediment and gas formation at the bottom of the sediment plates. Such phenomena in natural sediments are associated with anaerobic conditions. Neither sediment discoloration nor gas formation occurred within the treated sediments in this experiment. It was unlikely that there were no anaerobic or facultative bacteria in the sediments because the gas sterilization of sediment was not completely successful. It was assumed, therefore, that aerobic conditions were maintained in the 4 mm thick sediments despite the presence of bacterial populations.

Figure 12 - Bacteria Counts of the Sediment-water Mixtures for the Four Series. Vertical lines demonstrate ranges of three cultures. Sampling period means are connected.



Phosphorus Analysis--Medium-Biomass Phase

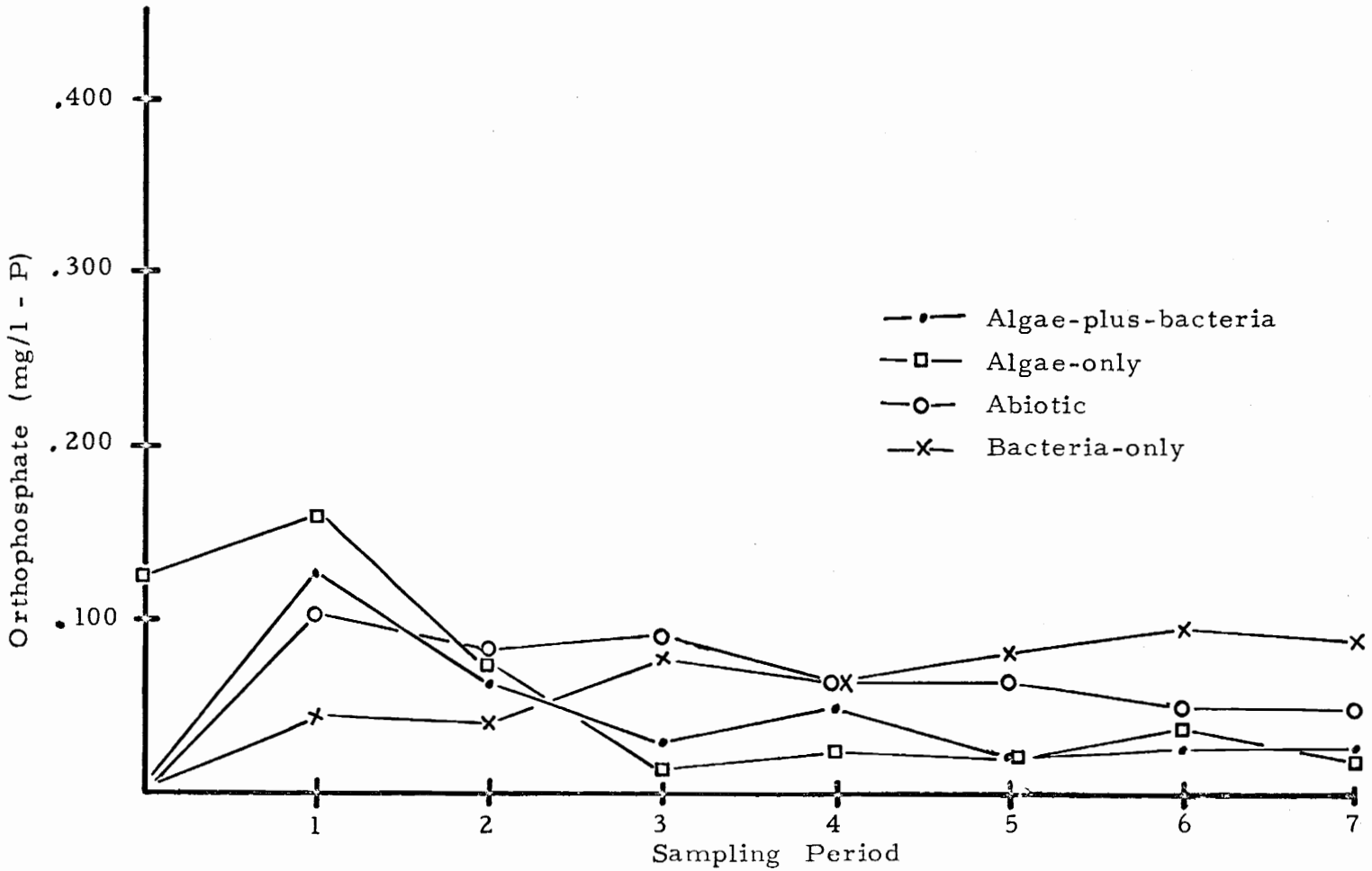
Orthophosphate

Orthophosphate phosphorus concentrations in the filtered medium for the four series are presented in Figure 13. Lines connect the means of three cultures in each series. The orthophosphate concentrations for uninoculated media are plotted at day zero. The high apparent orthophosphate concentration for the algae-only medium was credited to interference by the tellurite ion as previously mentioned.

It is evident that a considerable release of orthophosphorus from the sediments takes place within the first five days after inoculation. This initial release does not correlate with any significant biologic changes in the cultures. It, therefore, is ascribed to be the result of the establishment of a chemical equilibrium between aqueous and sediment inorganic phosphorus. Slight physical disruption of the sediment surface during introduction of liquid medium to the culture containers may affect the amount of exposed sediment surface, thereby increasing the amount of sediment phosphorus available to participate in the formation of the equilibrium. The differences in equilibrium concentrations between the four series at five days are not readily explainable.

In the first fifteen days of the experiment, orthophosphate concentrations in algae-containing cultures fell from an average of 0.195 mg/1 to as low as 0.015 mg/1 and remained below 0.052 mg/1 for the remainder of the experiment. The average orthophosphate concentration for the algal cultures on the last sampling date, 0.040 mg/1, is taken as the orthophosphate equilibrium concentration in growing algal cultures.

Figure 13 - Mean Orthophosphate Concentrations in the Media-biomass Phases after Dilution to 250 ml.



If, as intended, phosphorus was the limiting nutrient in the cultures, this equilibrium concentration would be the level at which orthophosphate uptake by algae would be equal to orthophosphate release from the sediments.

Average orthophosphate concentrations in the medium of the abiotic series show a constant decrease from 0.149 mg/l at five days to 0.070 mg/l at thirty-five days. Readsorption of orthophosphate onto sediment, adsorption onto the culture container, inaccuracy in analysis or any combination of these factors could explain the observed decrease. The possibility exists that the redox potential of the sediments was low when they were placed in the cultures. If this were so, the amount of orthophosphate released from the sediments in the first five days after inoculation would be higher than if the sediments were fully oxidized as intended. The decrease in orthophosphate concentration in the abiotic cultures may accompany a rise in sediment redox potential.

Orthophosphate concentrations in the medium of the bacteria-only cultures increase gradually from 0.062 mg/l at five days to 0.124 mg/l at thirty-five days in a manner that appears to be just the opposite of the abiotic cultures. Rapid adsorption of phosphorus by the bacteria followed by mineralization of organically-bound phosphorus is one explanation for this change. Another possible explanation is that bacterial metabolism enforced low redox potentials in the sediments, thereby solubilizing phosphate complexes and salts in the sediment. This would tend to raise the orthophosphate equilibrium concentration in the medium.

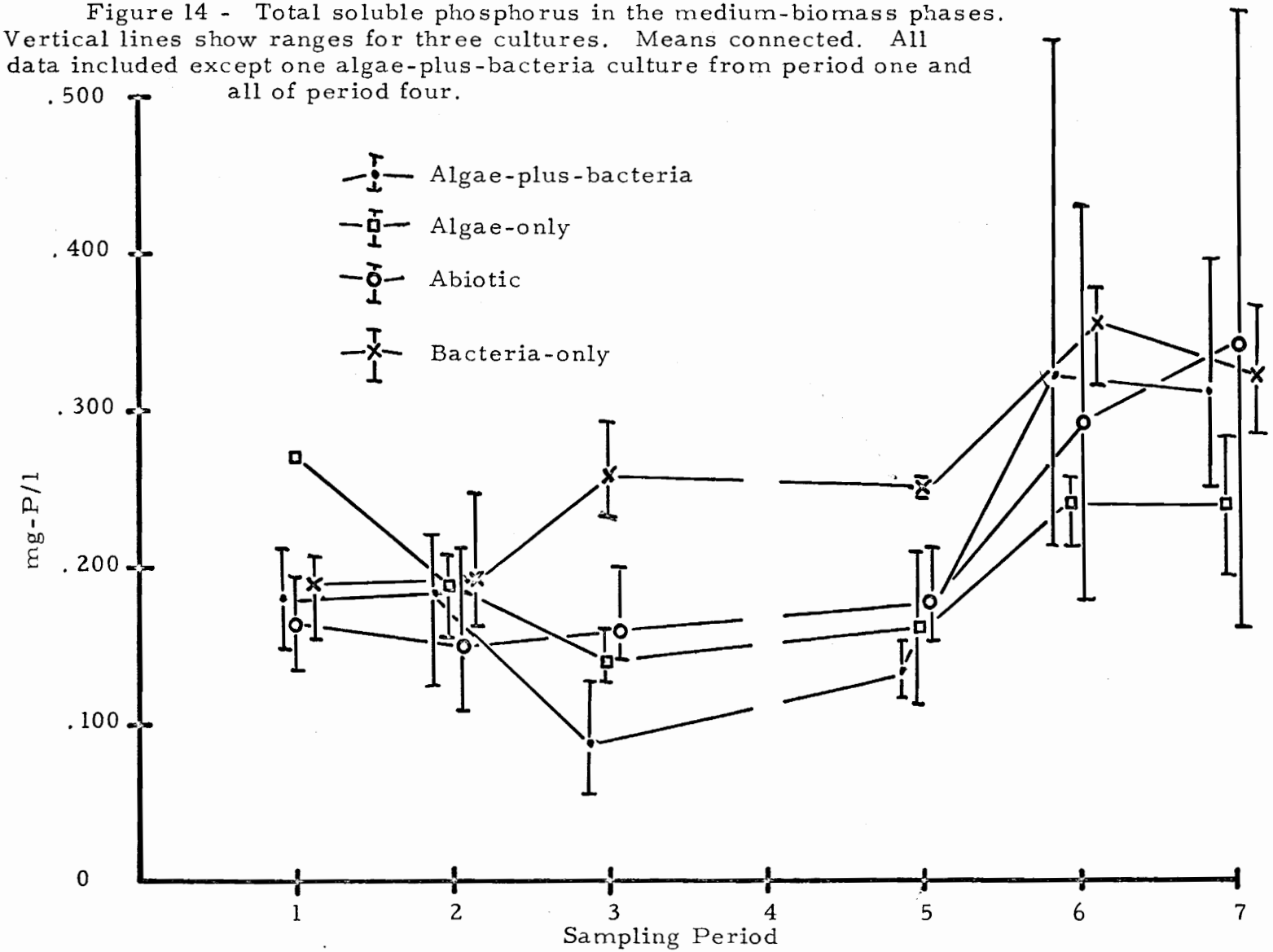
Total Soluble Phosphorus

Average total soluble phosphorus concentrations in filtered media in the four series is plotted in Figure 14. Erratic data, including all of the fourth sampling date data and one of the algae-plus-bacteria values for the first sampling date have been omitted from this figure.

The test for total soluble phosphorus appears to have been subject to considerable positive error. Relatively large ranges of values for sampling periods 1, 4, 6 and 7 are probably not explainable solely by differences between culture containers but are ascribed to be due, in part, to the separation technique, i.e., centrifugation followed by filtration of the medium. Small ranges of values for some of the sampling periods compared to ranges for other sampling periods may reflect varying refrigeration times between culture separation and phosphorus analysis.

The ranges obtained for total soluble phosphorus are too large in relation to the mean values for the four series to warrant identifying significant differences between the series. However, three observations have been based upon the data: (1) during the first two sampling periods, the values were similar for all series indicating minor biotic influences on total soluble phosphorus during this time; (2) starting with the third sampling period (fifteen days after inoculation), total soluble phosphorus in the bacteria-only series increased and remained slightly higher than the other series until the last two periods when the total soluble phosphorus in the several series appeared to converge. This apparent convergence may be an artifact

Figure 14 - Total soluble phosphorus in the medium-biomass phases. Vertical lines show ranges for three cultures. Means connected. All data included except one algae-plus-bacteria culture from period one and all of period four.

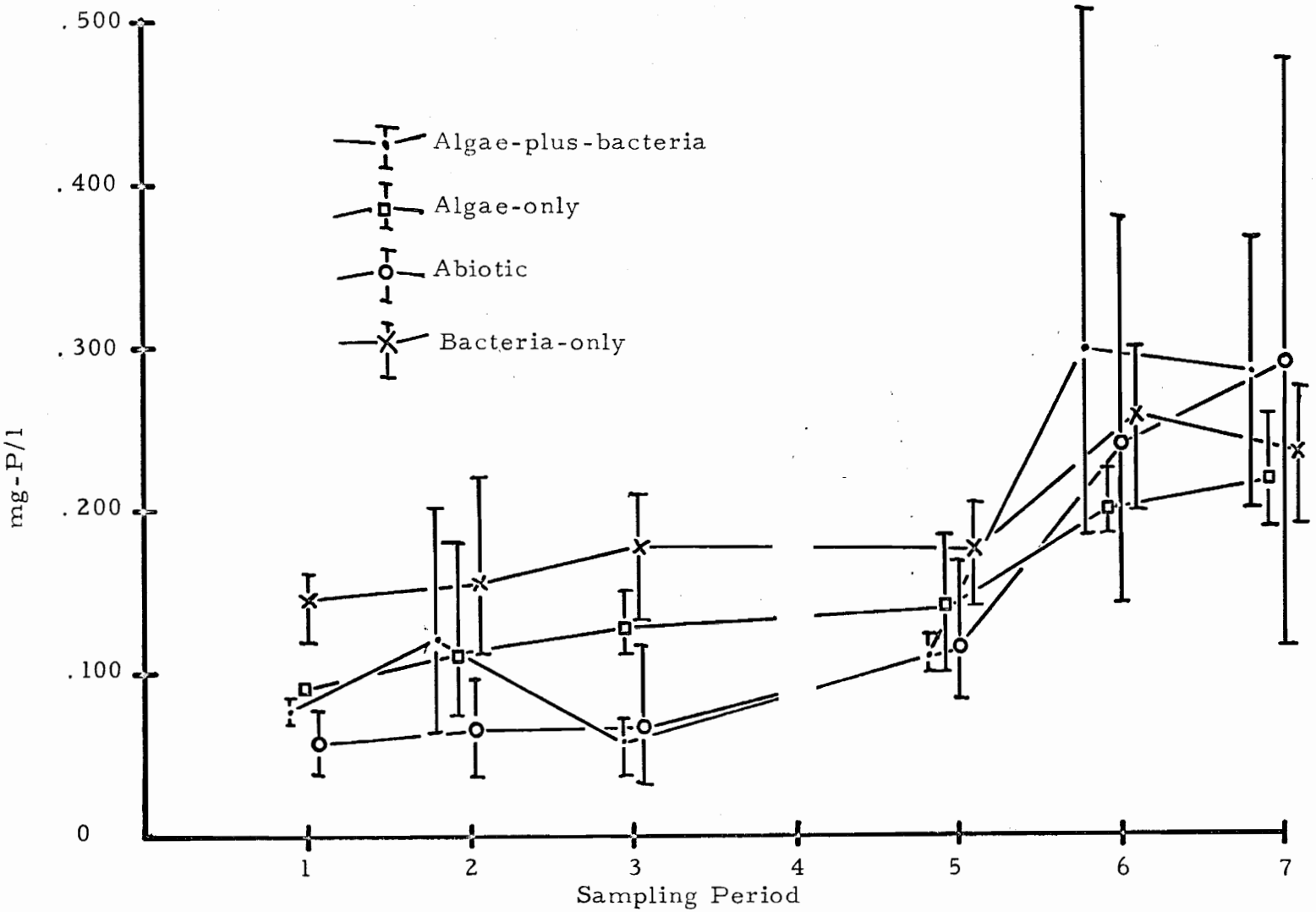


of positive errors in the last sampling periods; and (3) the mean values of total soluble phosphorus in the abiotic series for periods 1, 2, 3 and 5 and the lowest values for that series in periods 6 and 7 were all between 0.15 mg/l and 0.175 mg/l. This level of total soluble phosphorus is taken to represent the equilibrium due to physico-chemical processes for this parameter. From 30 to 79 percent of this phosphorus is orthophosphate-phosphorus.

Dissolved organic phosphorus was calculated as the difference between total soluble phosphorus and orthophosphorus. The values for dissolved organic phosphorus are presented in Figure 15. Variation in dissolved organic phosphorus within each series is nearly as great as variation between the series. Therefore, soluble organic phosphorus content is considered to be similar for all series. A gradual increase occurs from 0.05-0.15 mg/l at five days to 0.2-0.3 mg/l at thirty-five days.

As discussed by Rigler (8) the type of filter used to separate suspended and dissolved phosphorus fractions makes a significant impact on how much of the total dissolved phosphorus is determined to be organic. Filters with small, effective pore sizes were shown to pass smaller amounts of "dissolved" organic phosphorus than filters which would pass larger particles. It is possible in the present study that the 0.05-0.5 mg/l of "soluble organic phosphorus" was actually phosphorus bound to disrupted algal cells, bacteria and fine sediment particles. The similarity between series in the concentration of this parameter suggests that it is not actually phosphorus excreted by algae or bacteria.

Figure 15 - Soluble organic phosphorus in the medium-biomass phases. Vertical lines show ranges for three cultures. Means connected. All data included except one algae-plus-bacteria culture from period one and all of period four.



Total Centrifugable Solids Phosphorus

Figure 16 presents the mean values for total phosphorus bound to centrifugable matter as a function of time in the four series. The increase in this phosphorus fraction in the two algae-containing series was dramatic, especially in comparison to the changes displayed by the bacteria-only and the abiotic series.

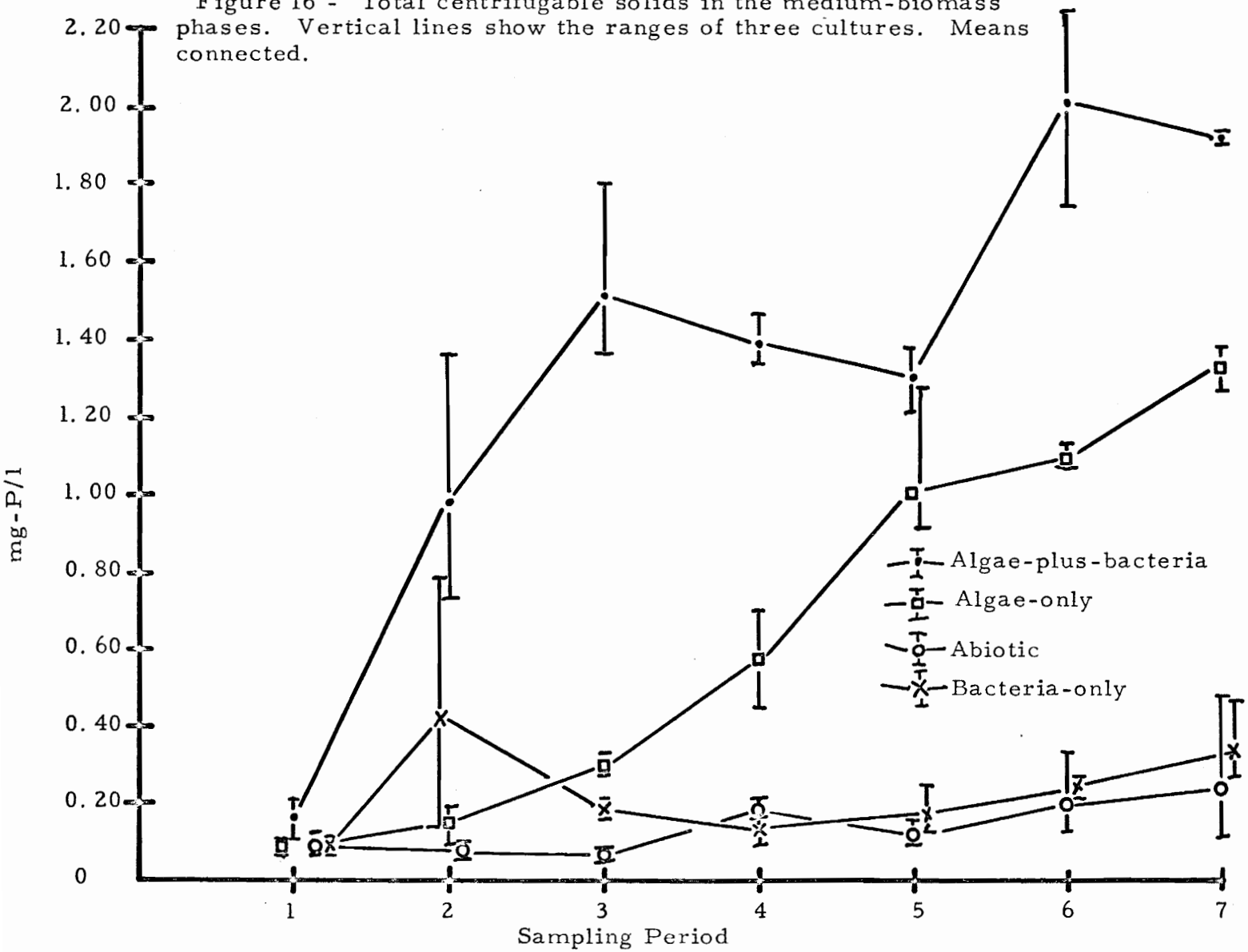
A source of error in these data was the inability of centrifugation to remove all organic particulate material. In the algae-containing series, especially the algae-plus-bacteria series, the intregity of the plug of centrifuged material was disrupted in some cases when the liquid was poured off and filtered for the other phosphorus tests. Algae retained on the filter was visible for some samples and represents a net loss of phosphorus from the liquid-biomass phase in calculating the phosphorus bound to centrifugable matter. Similarly, but perhaps more significantly, the amount of solid material removed by centrifugation from the bacteria-only cultures was very small and the plug was not cohesive. Bacteria not centrifuged out may have been lost on the glass fiber filters. No estimation of this loss was attempted. In retrospect, a more reliable method of estimating total phosphorus bound to particulate matter would have been to take the difference between total soluble (filtered) phosphorus and total phosphorus in an unprocessed aliquot of the medium.

Phosphorus Analysis--Sediment

Saloid-Bound Sediment Phosphorus

This fraction of the sediment phosphorus is the most likely source of both chemically and biologically available phosphorus in the

Figure 16 - Total centrifugable solids in the medium-biomass phases. Vertical lines show the ranges of three cultures. Means connected.



culture containers (27). Every culture's sediments were analyzed for this fraction. Saloid-bound phosphorus is reported in Appendix C as the weight of phosphorus in milligrams per 0.5 g of air-dried sediment and as the weight in the culture.

The literature consulted gave no mention of the amount of organic phosphorus that might be extracted with a weak aqueous-salt solution. "Saloid-bound phosphorus" appears to refer only to inorganic phosphorus. Therefore, the extracts obtained from the sediments for the first, fourth, and seventh sampling periods were digested and analyzed for total phosphorus. The results are presented in Table 4. Means and ranges of both inorganic and total phosphorus in the water extract for each series and sampling period are plotted in Figure 17.

The paucity of data for total phosphorus in the weak-salt extracts results in a negative error for estimating total sediment phosphorus. This error was calculated using data from the fourth sampling date reported in Appendix C. The error was -.67 percent for an algae-plus-bacteria culture and -.26 percent for an abiotic culture. Since there were only two cultures for which sufficient data were generated and since the error is smaller than the error in the analytical techniques used, no adjustments were made in the total sediment phosphorus values either reported in Appendix C or used in later calculations.

Inorganic saloid-bound phosphorus concentrations in sampling dates two through six show a general increase for all series. Confidence intervals for the test were not determined on the original, uncultured sediments, but the small ranges obtained for the series

TABLE 4

TOTAL PHOSPHORUS IN AQUEOUS-SALT
EXTRACTS OF SEDIMENTS

(Results in mg-P/Culture)

Culture	Sampling Period		
	1	4	7
I A+B	.0804	.0433	.0402
II A+B		.0306	
III A+B		.0253	.0375
I A	.1125		.0393
II A			
III A			.0504
I O	.1464	.0237	.0464
II O		.0134	.0278
III O		.0114	.0220
I B	.1500		.0317
II B			.0295
III B	.0804		.0288

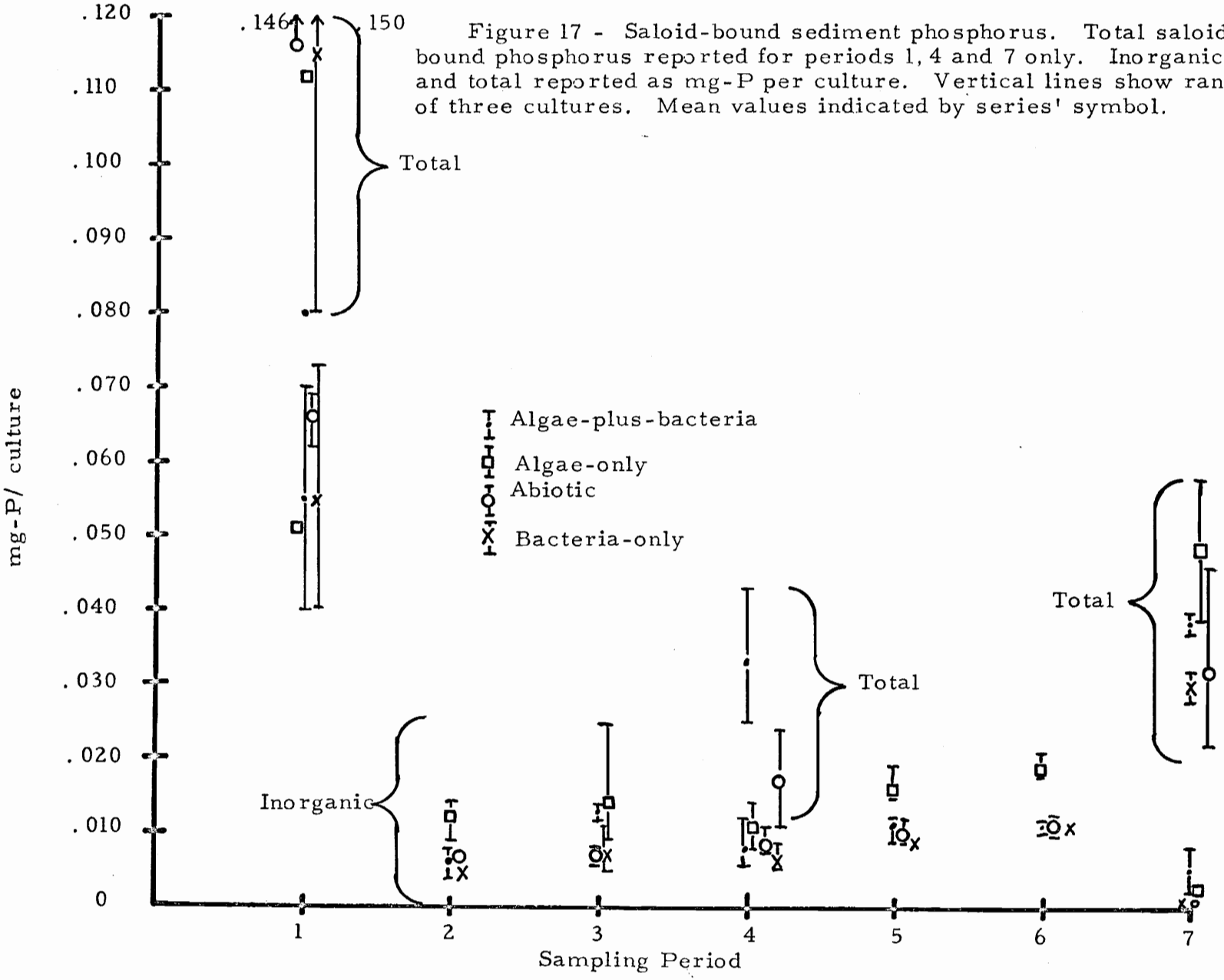


Figure 17 - Saloid-bound sediment phosphorus. Total saloid-bound phosphorus reported for periods 1, 4 and 7 only. Inorganic and total reported as mg-P per culture. Vertical lines show ranges of three cultures. Mean values indicated by series' symbol.

suggest a small variance for the analysis. However, significance of the upward trend cannot be evaluated.

The relative positions of the median inorganic saloid-bound phosphorus values are fairly constant for sampling periods two through six. The algae-only series has the highest median value in the five sampling periods; the bacteria-only series has the lowest median values in all but the sixth period; and the abiotic and algae-plus-bacteria medians are intermediate.

Results for sampling periods one and seven do not follow the even trend demonstrated for the other periods. The low values for period seven cannot be explained away on the grounds of analytical technique. In the absence of subsequent data, the period seven results were credited to sampling error or improper sample preservation. The results for period one were due to two mistakes. First, the sediment-water mixtures were allowed to stand at room temperature for two days prior to centrifugation and analysis. The odors produced in the two days indicated that the sediments had become anaerobic. Release of phosphorus due to the development of low redox potential is suspected. Secondly, no salt was included in the extract. Contamination by phosphorus of the NH_4Cl extract solution had been recognized shortly prior to culture separation and a substitute, NaCl, had not been evaluated in time. The results for period one compared with other periods illustrate the relative amounts of loosely-bound or interstitial phosphorus in sediments under anaerobic and aerobic conditions.

Inorganic and Total Sediment Phosphorus

Due to the complexity and lengthiness of the sediment extraction

and phosphorus analysis, not all of the culture's sediments were tested for phosphorus. Both concentrations (expressed as mg-P/.5 g sediment) and masses (expressed as mg-P/culture) of inorganic and total sediment phosphorus are presented in Appendix C. The mass of phosphorus in each culture's sediment was calculated by multiplying the phosphorus concentration by the air-dry weight of the sediment. Saloid-bound phosphorus was not included in values for inorganic and total sediment phosphorus.

Both inorganic and total sediment phosphorus concentrations are shown in Figure 18. Displayed along with this data are the means and 95 percent confidence intervals for total and inorganic phosphorus concentrations in the original sediments (includes saloid-bound phosphorus).

The erratic changes in sediment phosphorus concentrations, especially the total phosphorus concentrations, cannot be explained by the sediment extraction procedure or by the extract analysis method. The extraction and analysis were performed on the original sediments with much more uniform results. The lack of a recognizable trend in sediment phosphorus concentration for any of the series has made interpretation difficult and has left the results of the following statistical analysis in doubt.

Sediment phosphorus concentrations were determined for each culture that was separated in the sixth run. The hypothesis that the mean concentrations for the four series were equal to the concentration of the original, uncultured sediment was tested using the t-test for significance between two sample means (51). Table 5 presents the mean, standard deviation and "t" value for each series. At the 95 percent

Figure 18 - Total and inorganic phosphorus concentrations in Culture sediments. Reported as mg-P/ .5grams of air dried sediment. Values identified by series symbols. Vertical lines show ranges where applicable.

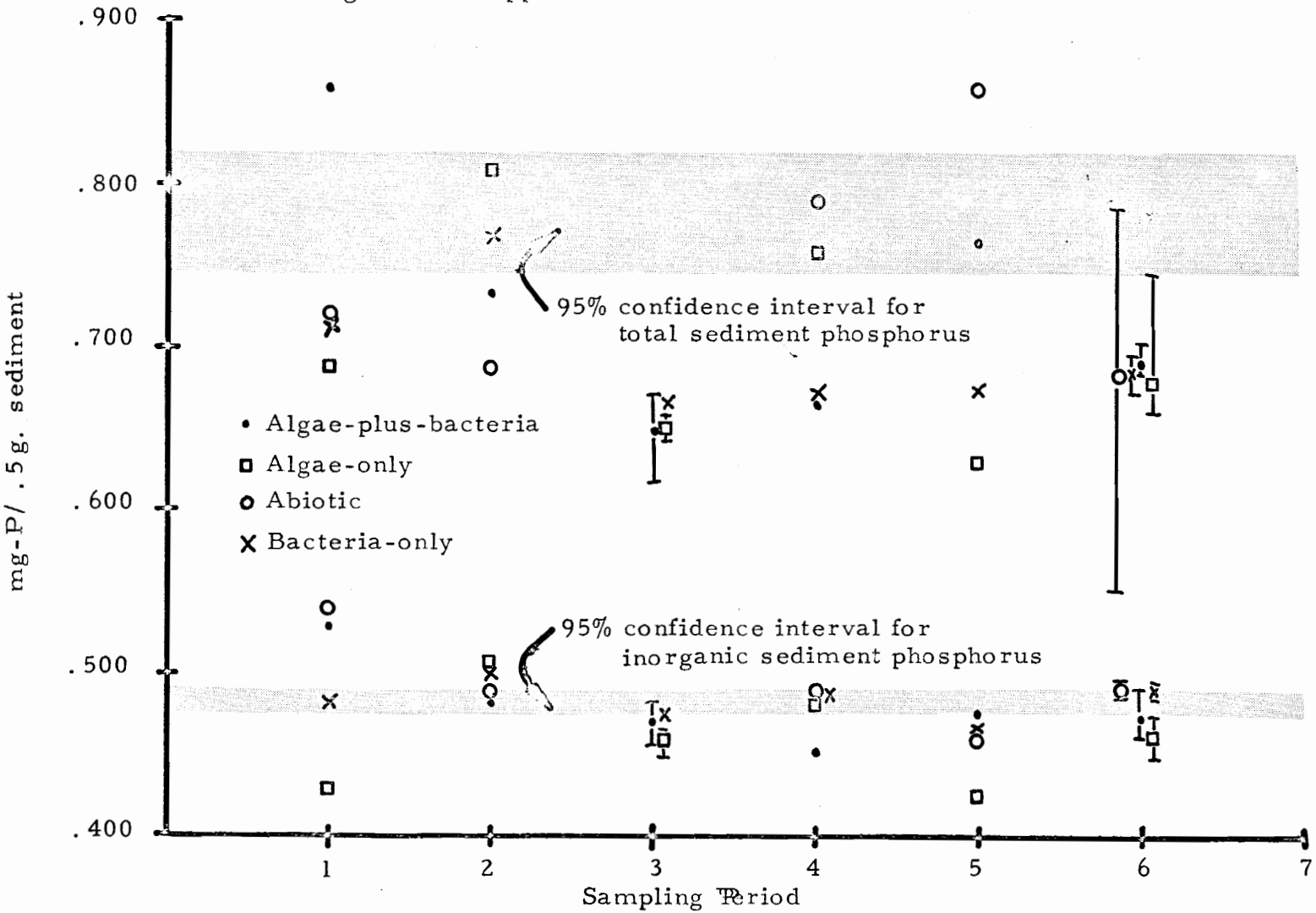


TABLE 5

SIGNIFICANCE OF THE DIFFERENCES BETWEEN
SIXTH-RUN CULTURE SEDIMENTS AND
THE ORIGINAL SEDIMENTS

Culture Series	Inorganic			Total		
	$\bar{x}$ mg-P/.5 g	s	"t"	$\bar{x}$ mg-P/.5 g	s	"t"
A+B	.472	.01688	-1.50	.687	.01225	-4.25
A	.460	.01360	-3.23	.677	.05933	-3.35
O	.491	.00387	1.92	.681	.12212	-1.98
B	.488	.00548	1.21	.681	.01095	-4.52
Original Sediments	.482	.00775		.782	.03682	

level of significance, values of "t" between -2.365 and +2.365 support the hypothesis that the means for the series and the original sediment are equal.

Only the algae-only series showed significantly lower values for both inorganic and total sediment phosphorus. Inorganic sediment phosphorus concentration dropped .022 mg-P/.5 g (4.6 percent) in the thirty-day period. Total sediment phosphorus concentration in this series dropped .105 mg-P/.5 g (13.4 percent).

In regard to inorganic sediment phosphorus, both algae-containing series showed declines in concentration over the thirty-day period. The two series from which algae were excluded, however, showed some increase in inorganic sediment phosphorus.

Whereas changes in inorganic sediment phosphorus were mixed and appear to be related to the presence or absence of algae, a consistent and larger decline of approximately .1 mg-P/.5 g (12.8 percent) in total sediment phosphorus was found in all series. This result contradicts the results for medium-biomass phase analysis which show that much less phosphorus was released to the liquid phase in the non-algae series than in the algae-containing series. Available data have not resolved this contradiction. Three possibilities that might explain the contradiction are:

1. Phosphorus actually released to the non-algae series' liquid phases might have been lost during the centrifugation filtration step of culture separation.

2. The actual loss of organic, saloid-bound phosphorus may be greater than the estimated .26 to .67 percent discussed in the previous section.

3. All of the sediment total phosphorus analyses may h been in error as suggested by the erratic values fo other sampling periods.

Dissolved Oxygen and pH

In order to evaluate the effectiveness of the Tris buffer and the aeration manifold in maintaining constant pH and dissolved oxygen, these parameters were measured in the last cultures sampled.

Hydrogen ion concentration was measured with a glass electrode. Values ranged generally from 5.8 to 6.8 with the exception of one inexplicable reading of 3.9 in one of the bacteria-only cultures. Differences in pH between the series were not noticeable. Since the pH of all culture media was originally adjusted to 7.0, the data suggest that the Tris buffer did not maintain its effectiveness.

Dissolved oxygen was measured by membrane electrode. Prior to aeration on the final day of culture, the algae-containing cultures had an average oxygen concentration 1.1 ppm above the saturation value for 20° C of 9.2 ppm. The abiotic and bacteria-only cultures had an average oxygen concentration .8 ppm below saturation. After one and one-half hours of aeration, the concentrations for all cultures approached a mean of 8.9 ppm.

DISCUSSION

The experimental results detailed in the previous section show that both short-term (less than five days) and long-term phosphorus exchange between sediments and water results in measurable transfer of phosphorus to the water. The results also show that the exchange increases when native microorganisms are present.

The experiments were not designed to determine the mechanisms involved in biological mediation of sediment exchange. The data do provide some insight into the mechanisms, however.

The amount and rate of phosphorus exchange, the effects of microorganisms on these amounts and rates, hypothesized mechanisms for this biological mediation and comparison of the present results to the findings of others are the topics of this discussion.

Figures 19 through 22 organize the medium-biomass phase phosphorus fractions for each series so that the changes are readily apparent.

Release of sediment phosphorus to the medium-biomass phase during the first five days of incubation was generally the same for all series: .065-.085 mg total phosphorus. The algae-containing cultures already showed the higher exchanges in this short time. Inorganic phosphorus in the medium ranged from .011 to .035 mg. The bacteria-only cultures had the lowest average inorganic phosphorus release.

Exchange rates for this first incubation period were calcu-

Figure 19 - Algae-plus-bacteria Series. Distribution of of Phosphorus fractions in the media-biomass phase.

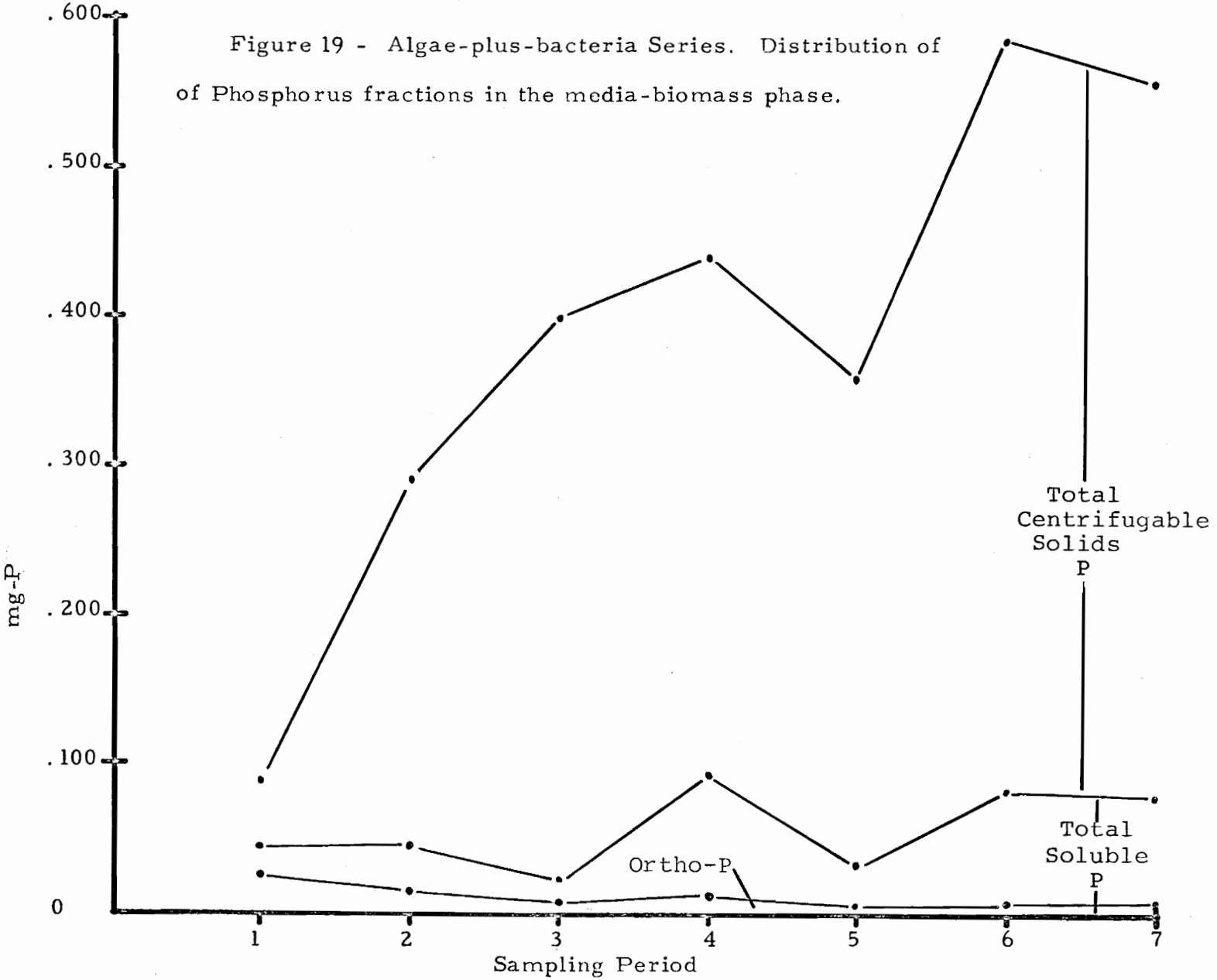


Figure 20 - Algae-only Series. Distribution of phosphorus fractions in the media-biomass phase.

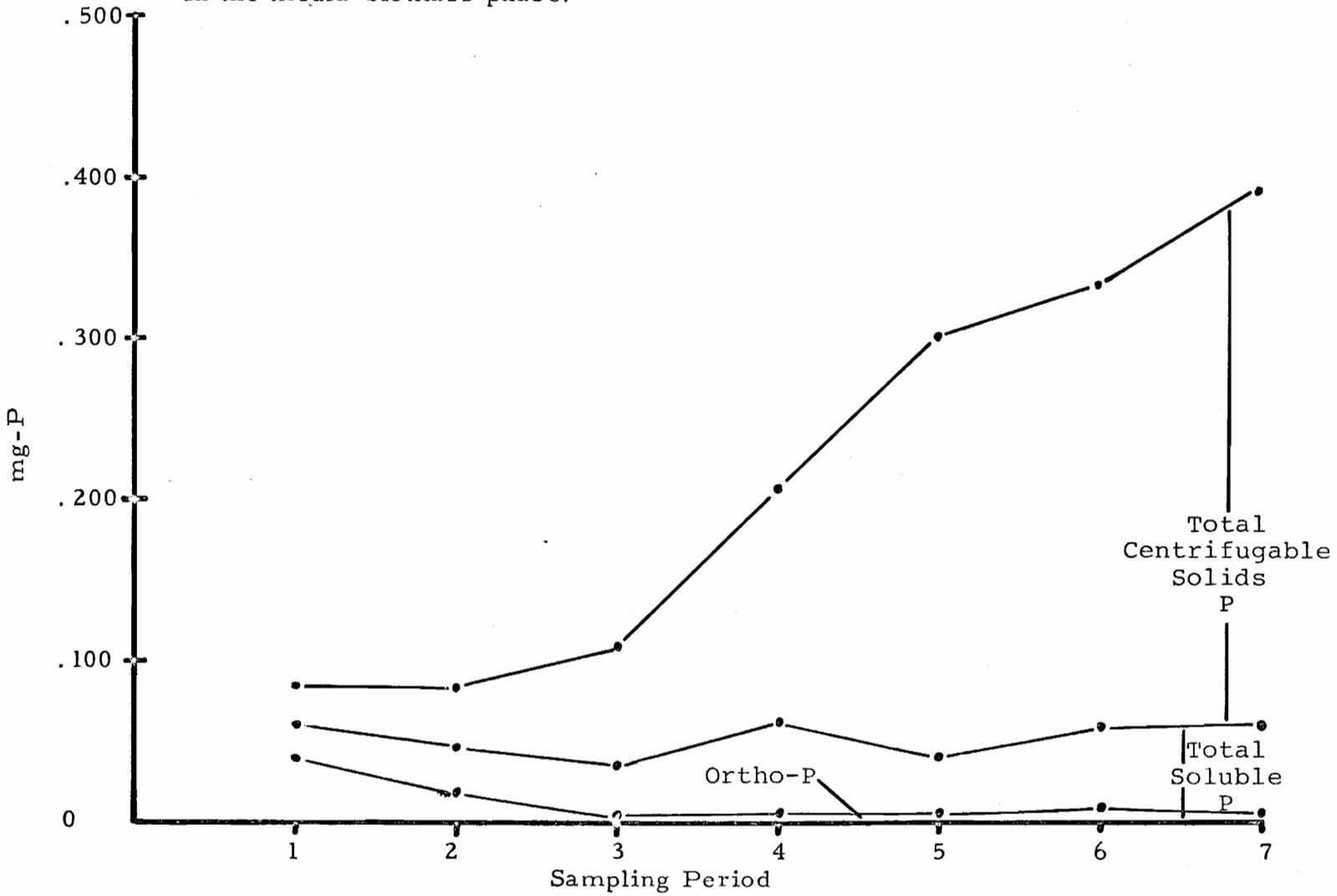


Figure 21 - Abiotic Series. Distribution of phosphorus fractions in the media-biomass phase.

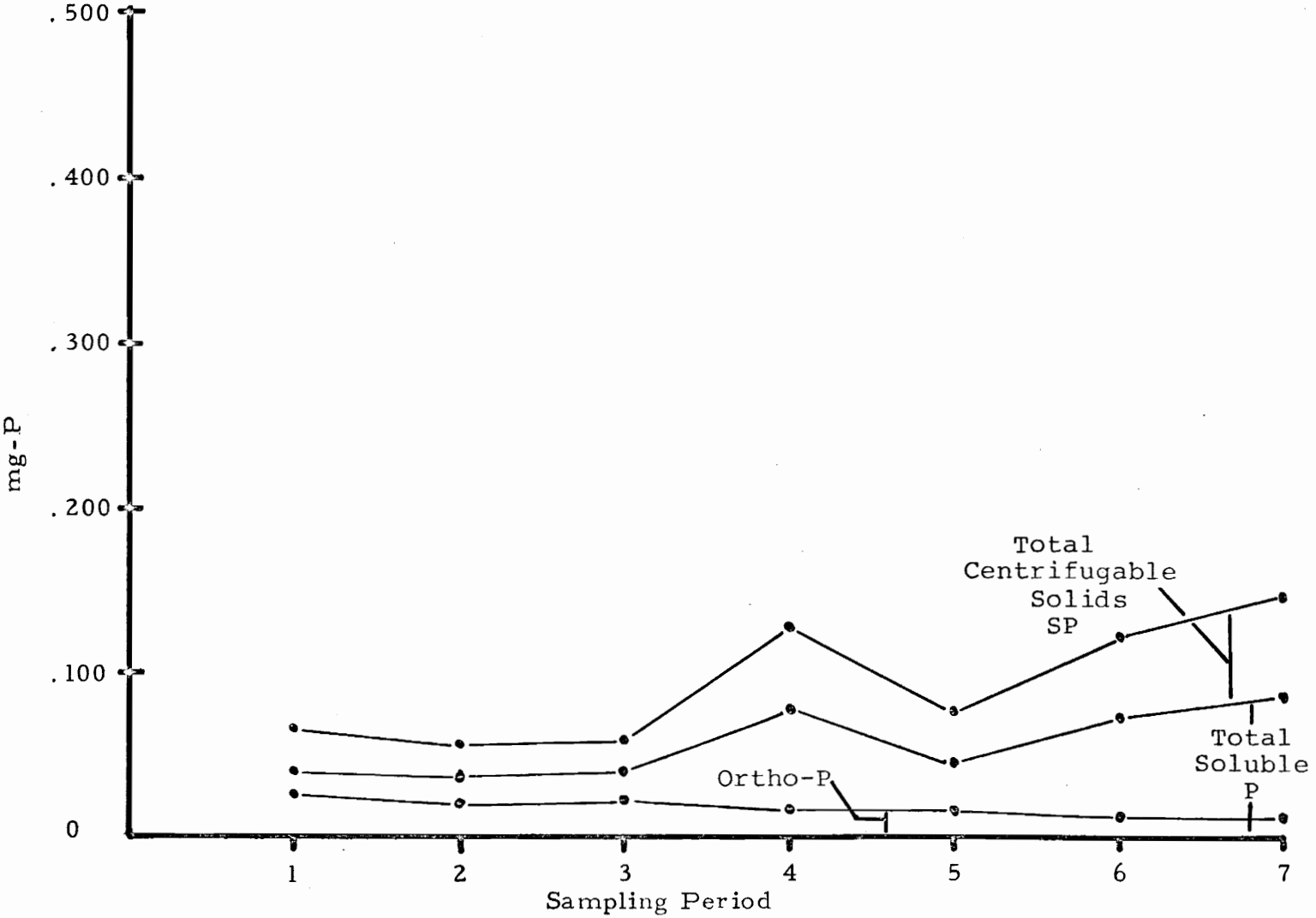
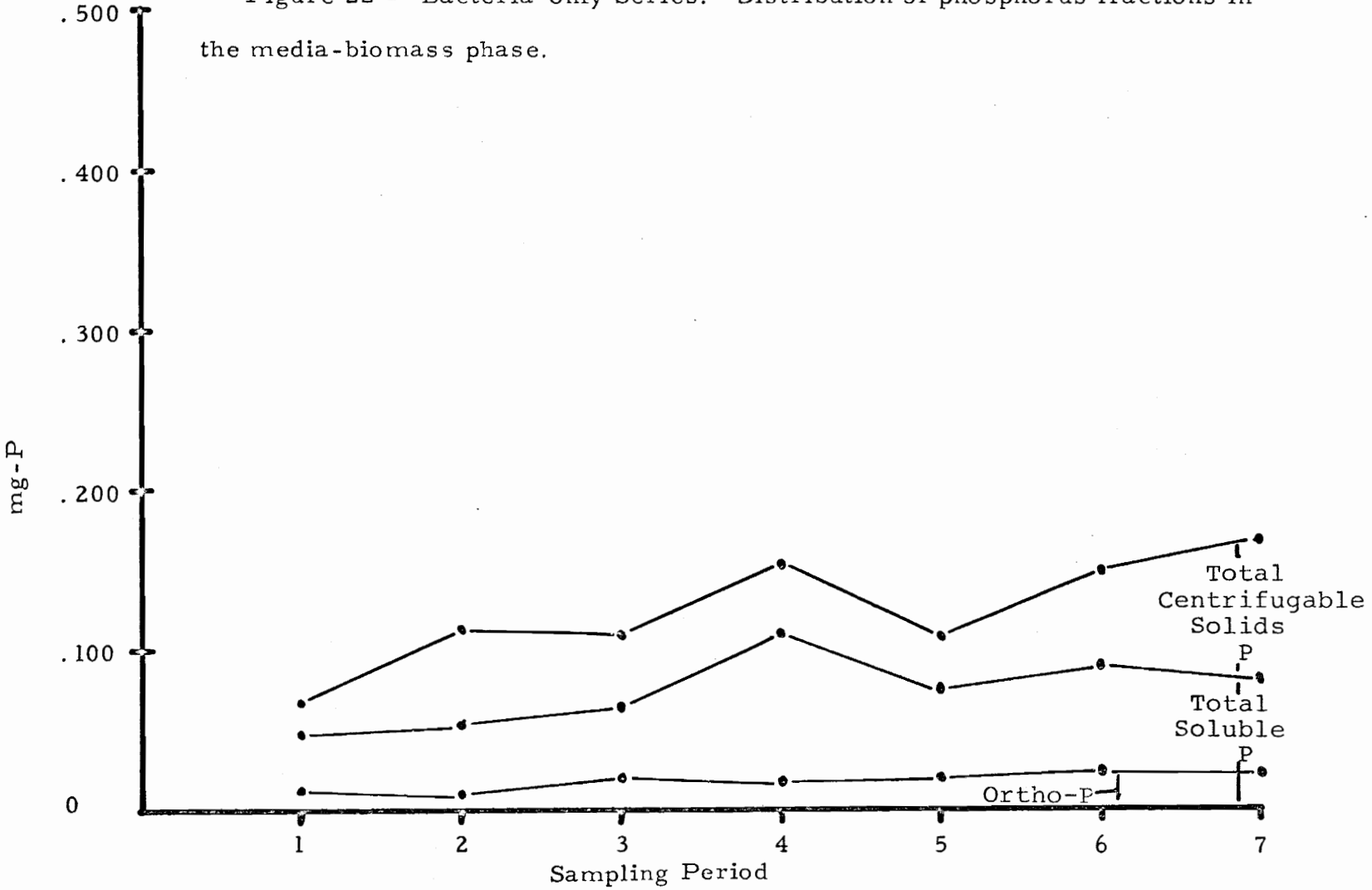


Figure 22 - Bacteria-only Series. Distribution of phosphorus fractions in the media-biomass phase.



lated using the average amounts of inorganic and total phosphorus in the medium-biomass phase for three cultures in each series. The sediment surface area for all cultures was 19.63 cm^2. These rates are presented in Table 6.

The inorganic exchange rates for the four series are 30 to 110 times as great as the exchange rate found for estuarine sediments by Pomeroy et al. (13) of 1 uM m^{-2} day^{-1}. The rates are four to twelve times Stumm and Leckie's (12) "maximum transfer rate" based upon diffusion of 9 uM m^{-2} day^{-1}. Stumm and Leckie's rate is more comparable to the present findings since they were derived from experimental fresh water systems. Diffusion alone, therefore, would appear to be a relatively minor component of the initial release.

Diffusion may be aided by other physicochemical processes. As noted in Appendix B, relatively small amounts, .9-1.6 mg, of sediment were displaced from the sediment plates (probably during culture preparation) and became a part of the medium-biomass phase. With a total phosphorus concentration of 0.782 mg/.5 g and an inorganic phosphorus concentration of 0.482 mg/.5 g, the displaced sediment could account for 1.6 to 3.8 percent of the total phosphorus and 2.2 to 12.8 percent of the inorganic phosphorus released during the first five days. Turbulent mixing would not appear to account for a significant proportion of the initial release. Actual mixing of the sediments, as would occur in natural sediments, did not take place in the cultures.

Stumm and Leckie (12) proposed sediment compaction as a possible cause of phosphorus release. If the sediments compacted from 4 mm depth to 3.5 mm (a compaction that could have gone unnoticed),

TABLE 6

PHOSPHORUS RELEASE DURING FIRST FIVE-DAY INCUBATION PERIOD[1]

	Inorganic-P			Total-P*		
	Amount mg	Rate mg/m^2day	Rate um/m^2day	Amount mg	Rate mg/m^2day	Rate um/m^2day
Algae-Plus-Bacteria	.025	2.54	78.7	.086	8.76	271.3
Algae-Only	.035	3.60	111.5	.085	8.65	268.2
Bacteria-Only	.011	1.113	34.5	.067	6.82	211.2
Abiotic	.014	1.40	43.4	.066	6.72	208.1

[1]Phosphorus release is assumed to be equivalent to increases in the phosphorus fractions in the medium-biomass phases.

*Total dissolved plus total centrifugable solids phosphorus

approximately one ml of interstitial water would have been displaced to the medium-biomass phase. Based upon the second sampling period, saloid-bound inorganic phosphorus amounts of .007 mg/culture (average for all twelve cultures) and assuming that 5 ml of the sediment volume (7.85 ml) was liquid, the one ml displacement could have released 0.0014 mg of inorganic phosphorus or approximately 10 percent of the inorganic phosphorus in the medium-biomass phases of the non-algae cultures. Dissolved organic and particulate phosphorus could have been released in the same proportion.

Diffusion, mixing, and compaction account for only 40 to 50 percent of the initial phosphorus release in the non-algae series. The remainder of the initial release may be accounted for by analytical error or by possible accelerated release due to low oxidation-reduction potentials in the sediments. Available data do not allow evaluation of these potential causes.

Phosphorus exchange between days five and thirty-five varies considerably among the four series. The differences between series suggests biological mediation of phosphorus exchange.

If it is assumed that only the inorganic phosphorus that is released affects surface water productivity, then examination of inorganic phosphorus release by itself would be valid. However, other forms of phosphorus, that is, dissolved organic and particulate, can either be released directly to surface waters or are formed when released inorganic phosphorus is adsorbed biologically. Dissolved organic phosphorus and particulate phosphorus are not readily utilizable by algae or plants. They do represent potential sources of

available phosphorus after mineralization by bacteria or through enzymatic hydrolysis of organic phosphorus compounds by phosphatases (52, 53). The non-inorganic phosphorus compounds also represent a substantial fraction of the gross phosphorus released from the sediments in the present experiments. Some of these non-inorganic compounds appear from the data to have been released directly--the rest were the end products of biological assimilation of released inorganic phosphorus.

Phosphorus releases between five and thirty-five days are presented in Table 7. The amounts and rates of phosphorus release are based upon net change between five and thirty-five days in inorganic and total phosphorus in the medium-biomass phase. It is immediately apparent that interpretation of inorganic release (which appears to be negative for all series except "bacteria-only") would yield altogether different conclusions from interpretations of total phosphorus release.

Long-term inorganic phosphorus release was discussed previously (see "Results"). The negative long-term release rates as reflected by changes in its concentration in the medium-biomass phase do not reflect the actual release of inorganic phosphorus. The decreases in concentrations have been attributed to rapid uptake by growing algae (algae-containing series) and to readsorption by the sediment (abiotic series).

Overall release of phosphorus as reflected by increases in total phosphorus in the medium-biomass phases appears to be strongly influenced by algae. Algae-containing series had rates of total phosphorus increase three to six times greater than non-algae series.

There are four mechanisms by which algae might increase the transfer of phosphorus from sediments. The most significant mechanism

TABLE 7

PHOSPHORUS RELEASE FROM DAY FIVE TO DAY THIRTY-FIVE[1]

	Inorganic-P			Total-P*		
	Amount mg	Rate mg/m^2day	Rate um/m^2day	Amount mg	Rate mg/m^2day	Rate um/m^2day
Algae-Plus-Bacteria	-0.0256	-.435	-13.5	0.471	7.99	247.5
Algae-Only	-0.0354	-.600	-18.6	0.306	5.19	160.8
Bacteria-Only	0.0109	.185	5.7	0.101	1.71	53.0
Abiotic	-0.01375	-.233	-7.2	0.020	1.36	42.1

[1]Phosphorus release is assumed to be equivalent to increases in the phosphorus fractions in medium-biomass phases.

*Total dissolved plus total centrifugable solids phosphorus

is based upon algae's ability to act as a phosphorus "sink." Orthophate exists in equilibrium between sediment and overlying liquid. Pomeroy et al. (30) and Hayes and Phillips (18) showed that algae rapidly sorb orthophosphate. This would effectively remove it from participation in the equilibrium. To restore the equilibrium, sediments release orthophosphate. If the equilibrium is to be maintained, sediments must release phosphorus at the same rate as growing algae sorb and store it. In these experiments the orthophosphate concentration in algae-containing cultures declined indicating that release from the sediments was not keeping pace with algal uptake.

Porcella et al. (30) found that mats of Oscillatoria developed anaerobic conditions with associated purple sulfur bacteria between the mat and sediments. The anaerobic conditions likely accelerated release of phosphorus in Porcella's cultures. No purple sulfur bacteria were visible in any part of the sediments in the present experiments. While no redox potentials in the sediments were measured, it was thought that the sediments remained aerobic.

The algae may release enzymes which facilitate the hydrolysis of organic phosphorus, thereby shifting the orthophosphate equilibrium in favor of the algae. This might not occur in sediments due to inactivation of extracellular phosphates as a result of enzyme sorption on sediment particulates (54) [cited in Syers et al. (5)]. Many algae have been shown to have intracellular phosphatases (55). Motile algae possessing intracellular phosphatases could acquire phosphorus from sediments without depending on orthophosphate equilibria. There is no evidence to indicate that this has occurred in these experiments.

Lacking sufficient data to support other mechanisms, it is hypothesized that the algae acted simply as a phosphorus sink.

It is doubtful that the blue-green algae were phosphorus limited. Orthophosphate concentrations at the end of the experiment (before culture separation and dilution) ranged from 0.017 to 0.044 mg/1. Sawyer and McCarty (56) state that .01 mg-P/1 is sufficient to support algal blooms. These concentrations are representative of the optimum growth range for a variety of algal species (57, 58) [cited in Fogg (55)].

The inorganic phosphorus concentration in the medium-biomass phase was much smaller than the concentration of interstitial (more correctly "saloid-bound," considered here to be nearly equivalent) phosphorus in the sediments. Estimating that approximately 5 ml of the 8.5 ml volume of sediments was interstitial water, the concentrations of interstitial phosphorus were calculated to range from .8 to 4.2 mg/1. These values fall within the range of .08 to 10.5 mg/1 reported for various eutrophic lakes in the western United States (59). The values were well above the .06 to .15 mg/1 reported for the less eutrophic Lake Constance (60). The interstitial inorganic phosphorus concentration varied from twenty-five to 125 times the inorganic phosphorus concentration in actively growing algae cultures. In bacteria-only and abiotic cultures the ratio was roughly 10-50 to 1.

Pomeroy et al. (13) found that the estuarine sediments that they studied were in phosphate equilibrium with water having an inorganic phosphate content of 0.7 to 0.9 um/1 (0.023 to 0.029 mg-P/1). This is within the range of orthophosphate concentrations in algae-containing

cultures at the end of the present experiment, 0.017 to 0.044 mg-P/1, but is somewhat less than the 0.038 to 0.136 mg-P/1 remaining in the non-algae cultures (bacteria-only: 0.107 to 0.136 mg-P/1; abiotic: 0.038 to 0.108 mg-P/1).

The role of bacteria in phosphorus exchange appears from data in the present experiment to be minor. Bacteria are generally thought of as mineralizers. The increase in orthophosphate concentrations in the bacteria-only series supports this. The bacteria may have made sediment phosphorus available to algae in the algae-plus-bacteria series more rapidly than equilibrium mechanisms alone. Supporting this line of thinking is the apparent reduction in algal growth rate when bacterial growth was inhibited (algae-only series). However, the need for bacteria to provide orthophosphate for algal growth is not apparent--orthophosphate concentrations are adequate for rapid algal growth in all cultures regardless of whether bacteria are inhibited or not.

Bacteria may play a special role in the growth of blue-green algae that is not connected with phosphorus availability. Bacteria are often intimately associated with the cell walls and mucilaginous excretions of blue-green algae. These bacteria may provide growth factors required for algal growth. Temporary inhibition of such bacteria by potassium tellurite in the algae-only series may have resulted in the observed slow initial algal growth rates even in the presence of adequate amounts of orthophosphate. This hypothesis is not proven by available data. Resolution of the effect of tellurite on algal growth and phosphorus uptake would depend upon culturing the subject algae in axenic, non-sediment cultures with appropriate concentrations of potassium tellu-

rite. This was not possible in the time available.

Bacteria alone have negligible capability in accelerating phosphorus release from sediments. This is evidenced by only slightly greater total phosphorus concentrations in the bacteria-only series than in the abiotic series.

Application of the results reported here to the prediction of phosphorus release rates in natural conditions is risky. By design, many factors which influence phosphorus exchange (transfer into as well as out of) between sediment and water have been eliminated. Also, the length of the experiment was insufficient to determine phosphorus transfers in systems at biological equilibrium--all algae cultures, for instance, were apparently still in the growth phase when the experiment was terminated. Therefore, the phosphorus transfer rates for the several series must be interpreted for what they are: maximum, uni-directional rates representing only one portion of a complex phosphorus exchange pattern.

Stumm and Leckie (12) discussed R. Vollenweider's correlation between areal phosphate loading and degree of enrichment. By Vollenweider's correlation, a lake fifty meters deep would be classified eutrophic if it received 500 mg-P per square meter per year. This is equivalent to 1.37 mg-P per square meter per day. All of the series in the experiments reported here had release rates of this size and larger (see Table 7).

It would be inappropriate to claim that the observed phosphorus release rates show that the Occoquan Reservoir will remain eutrophic if the sediments become the sole source of phosphorus to the surface water.

As discussed in the introduction, nutrient availability is not directly definitive of eutrophication. The profuse algae growth supported by sediment phosphorus does contribute to the two indices of eutrophication cited in the introduction: decrease in lake volume and increase of stored energy in the lake system.

In regard to nutrient availability, it is appropriate to state that nutrients removed from the sediments that end up in the form of particulate and dissolved organic phosphorus are potentially available for further primary productivity in surface waters or on the sediment. Grazing of attached algae by benthic or planktonic herbivores or decomposition by bacteria can be expected to transfer the phosphorus, once contained in the sediment, to any other part of the lake system.

CONCLUSIONS

1. Aerobic surficial sediments can serve as a phosphorus source for the growth of blue-green algae.

2. Under laboratory conditions, aerobic sediments from the Occoquan Reservoir released phosphorus in the presence of the following types of microorganisms at the rates below:

	Initial	Long-Term
Blue-Green Algae Plus Bacteria	8.76	7.99 mg/m^2 day
Blue-Green Algae Only	8.66	5.19
Bacteria Only	6.82	1.71
Abiotic	6.72	1.36

3. Algae appear to act as a phosphorus sink. The presence of this sink greatly accelerates the release of inorganic phosphorus from sediments.

4. Bacteria by themselves appear to have little direct effect upon overall release of phosphorus from sediments. They do appear to mineralize organically bound phosphorus to increase the orthophosphate concentration in the liquid phase.

5. Phosphorus release rates as high or higher than the "initial" release rates might be expected in the Occoquan Reservoir whenever inorganic phosphorus concentrations in the water contacting the sediments fall substantially below .07 mg/1. This is the approximate peak

orthophosphate concentration (undiluted) reached in abiotic cultures in the first five days of incubation.

APPENDIX A

SEDIMENT STERILIZATION

Ethylene oxide sterilization of sediments was attempted in this study in order to: (1) avoid the alteration of organic phosphorus fractions that would have been caused by heat (autoclaving) or oxidation (ultraviolet light); and (2) preclude toxic residuals in the sediment (formalin, mercury).

Specific advantages of ethylene oxide treatment were:

1. At room temperature, ethylene oxide is a gas with exceptional ability to penetrate other materials (61, 62, 63). In sediments that have high organic and inorganic solids, the ability to disperse rapidly would promote sterilization.

2. As an alkylating agent, ethylene oxide was not felt to be able to liberate organically bound phosphates. Oxidants, heat, and ultraviolet radiation could all be expected to release organic phosphates as orthophosphates.

3. No toxic residues were expected to be left in the sediments. Ethylene oxide either would react chemically with the substrate to form non-toxic compounds or would diffuse out of solution as the gas.

Disadvantages of using ethylene oxide were:

1. In aqueous solutions, ethylene oxide is converted to ethylene glycol, which may be used as an organic substrate for heterotrophic metabolism. The amount of organic substrate added to sediments and the ability of bacteria to utilize it have not been determined.

2. Ethylene oxide at concentrations of 3 percent or more in air is highly explosive. As an alkylating agent, it can cause skin and lung burns. Its use requires good ventilation and constant awareness of its danger in design and operation of sterilization equipment.

3. Ethylene oxide's effectiveness is greatest at 93 percent relative humidity so that some moisture is required. However, its actual effectiveness in aqueous solution with a high solids content is not reported in the literature. Ethylene oxide has been reported to be bactericidal in milk (64).

4. Ethylene oxide may alter the form of some organic phosphorus compounds. Stacey (65) reports that epoxides such as ethylene oxide react with DNA to esterify the phosphate groups.

5. The chemical may destroy some organic algal nutrients. Thiamine is destroyed by ethylene oxide (66). However, Vitamin B_{12} is not affected.

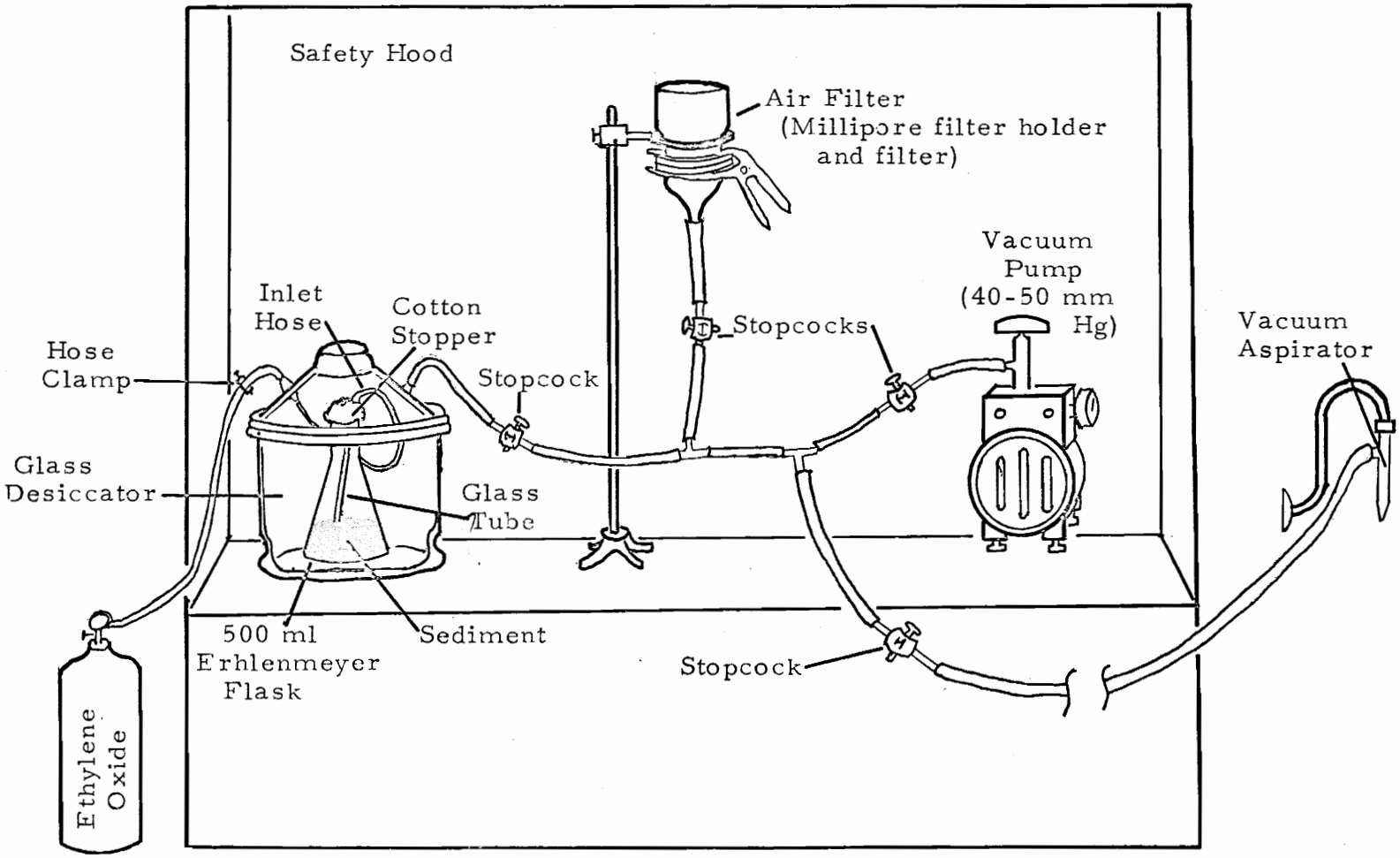
The apparatus designed for treating the sediments with ethylene oxide is shown in Figure A-1. The gas chamber was a large, glass desiccator. Two holes drilled in the solid glass cover were fitted inside and outside with glass tubing nipples. Ethylene oxide was supplied from a cylinder of the compressed gas through rubber tubing to the inlet nipple. Another section of tubing inside the gas chamber conducted the gas to a 500-ml Erlenmeyer flask containing the sediment. The tubing was attached to a glass tube with drawn tip inside the flask, the tip being submersed in the sediment. A cotton plug in the neck of the flask held the glass tube in place and prevented recontamination of the sediments after treatment while allowing ethylene oxide to diffuse out.

A vacuum pump, vacuum aspirator, and air filter were attached as shown to the outlet nipple of the gas chamber. Appropriate glass stopcocks were included to allow sediment treatment by the following routine:

1. Using the vacuum pump, the gas chamber was evacuated to an estimated 40-50 mm Hg.

2. Ethylene oxide was slowly introduced into the chamber by bubbling it through the sediments. The gas supply was immediately turned off when the pressure had built up enough to allow the desiccator lid to be slid sideways.

Figure A-1 - Apparatus for the Ethylene Oxide Treatment of Lake Sediments.



Some additional mixing of the sediments was provided by a magnetic stirring bar and stirrer during this step.

3. The ethylene oxide-saturated sediment was allowed to stand for eight to twelve hours.

4. The chamber was alternatively evacuated using the vacuum aspirator and then refilled with air filtered through a .45 micron HA Millipore filter. This was repeated at least four times and the vacuum was held each time for fifteen to thirty minutes to rid the chamber and sediment of ethylene oxide.

The success of the ethylene oxide treatment was tested using: (1) an aqueous bacterial culture; and (2) samples of the sediments to be used. After treatment, 0.1 ml of each were plated on Pond Water Agar and incubated at 20° C. After seven days, the plated aqueous bacterial culture showed no growth, indicating a successful sterilization. After four days, the plated sediments, however, had 100-250 colonies per plate compared to a bacterial lawn produced when untreated sediments were plated.

Data on bacterial growth in culture sediments gathered in this study support this finding that the sediment sterilization was not completely effective. A substantial reduction in bacterial numbers was achieved so that the term "pasteurization" might be more appropriate than "sterilization."

APPENDIX B

ALGAE CONSIDERATIONS

Several topics related to algae types and algae growth were important to the design and results of this study. These topics are discussed separately here and include: (1) choice of the algal inoculum; (2) algal species in the cultures at the end of the experiment; (3) suspended solids, chlorophyll and phosphorus relationships at the end of the experiment; and (4) growth of the algal inoculum in non-sediment cultures.

Choice of the Algal Inoculum

Three taxonomic groups of algae were considered likely to be able to utilize sediment phosphorus: Cyanophyta (blue-green algae), Bacillariophyceae (diatoms), and Volvocaceae (motile, coenobic, green algae). All three groups are represented by species which are abundant in Occoquan sediments. All three groups have motile species which could transport from the sediments to overlying water the phosphorus incorporated in their cells.

A Volvocacean species, thought to be of the genus Eudorina, was the first alga to grow and the only species to form a bloom in preliminary experiments. These experiments involved incubation of untreated sediment samples in an algal medium (Graham's ASM-1 used in the main experiments) from which phosphorus had been excluded. This growth indicated the algae's ability to readily utilize sediment phosphorus.

Diatoms were considered likely to be able to utilize sediment phosphorus for two reasons. First, they have a nutritional requirement for iron (67). If ferric phosphates precipitated in the sediment could serve as an iron source for diatoms, the phosphate released when iron is split off might also be used by the diatoms. Secondly, they have been shown to follow a diurnal migration into and out of sediments (68). This migration would bring the cells into contact with relatively large amounts of sediment during dark periods while allowing for maximum photosynthetic activity during light periods.

Blue-green algae, commonly associated with eutrophic conditions, have been shown to be able to produce phosphatases (52, 53, 55), enzymes which split phosphates off of organic molecules, thereby making the phosphates available to the algae. In eutrophic lakes such as the Occoquan Reservoir, a significant proportion of the phosphorus in sediments is tied up in organic compounds. Algae which could utilize this source of phosphorus may have a competitive advantage over algae that must depend upon orthophosphates alone.

Blue-green algae were chosen to be the group used as the algal inoculum because of: (1) the ease of using inhibitors (Actidione) to eliminate other algae groups; and because (2) the blue-green algae species that were recognized microscopically in the sediments and cultured in preliminary experiments were mat-forming (Oscillatoria) or colonial (Nostoc, Anabaena, Gleocapsa) algae which had potential of being easily separated from sediments.

Algal Species in the Cultures

One algae-plus-bacteria sediment culture and one algae-only

sediment culture were taken out of the final sampling at thirty-five days and examined for the types of algae present. Microscopic examination of algae from various parts of the cultures was performed with a Wild Heerburg inverted microscope calibrated with a Whipple disc occular and a slide micrometer.

Common to both cultures were two species of Oscillatoria which were very similar to each other. They may, in fact, be the same species showing physiological changes under differing conditions. Oscillatoria subtilissima had very faint cross walls, the diameter was less than 2 um, the cell length was about two and one half times the diameter, the trichomes were round and blunt on the ends, and the color was a pale, yellow-green. This alga was present in a transparent matrix adhering to the container walls just below the liquid surface of both cultures and was particularly abundant in the algae-plus-bacteria culture.

Also present in both cultures, and more abundant in the algae-plus-bacteria culture, was Oscillatoria limnetica. This alga had more distinct cross walls than Oscillatoria subtilissima, had a slightly greater diameter of 2.0 to 2.5 um and was noticeably greener. The ratio of cell length to diameter and the shape of end cells are identical in both algae. Oscillatoria limnetica was limited in distribution to the mat which developed on and under the nylon screen and on the container bottom. The occurence of two such similar forms together in the same culture with a distinct but intergraded zonal distribution suggests that the species are not two but one and that the variation observed was due to nutritional, not specific, differences.

Particularly abundant in the algae-only culture but rare in the algae-plus-bacteria culture was the distinctive species Oscillatoria grunowiana Gomont var. articulata. Cells of this species are longer than wide and have characteristically thick, transparent cell walls. The end cells were rounded and were not attenuated. This alga was found under the nylon screens of both cultures and was predominant in the algae-only culture. It was abundant also in the mat on the bottom of the algae-only culture container.

Scarce, but evenly distributed in both cultures, was the large Oscillatoria subbrevis. Five to 6 um in diameter with cells shorter than their diameter, this was the only Oscillatoria to have the dark, blue-green color commonly associated with blue-green algae.

Relatively abundant in the bottom of the algae-only culture, but rare elsewhere, were representatives of the Family Chroococcaceae and the Family Nostocaceae. The chroococcaceaen algae were thought to be of either the genus Aphanocapsa or Gleocapsa. The nostocacean algae were thought to be of either the genus Anabaena or Nostoc. Identification of these algae was hampered by the presence of sediment and masses of other algae. The reasons for their abundance in the algae-only culture and their rarity in the algae-plus-bacteria culture have not been identified.

Despite the use of the eukaryote inhibitor, Actidione, a variety of eukaryotes were present in these thirty-five day old cultures. A little, round, green alga with a diameter of 1.5 um was found in both cultures in the transparent matrix adhering to the container walls below the top of the liquid media. It was particularly abundant in the algae-

plus-bacteria culture and seemed to be distributed similarly to Oscillatoria subtilissima in both cultures. No organelles could be seen in this algae at 1500 X.

Ciliated protozoans and a few amoebae were found in both cultures. The protozoans and a highly motile, irregularly shaped organism possessing dark organelles were abundant in the algae-only culture.

No algal growth was seen in either the bacteria or the abiotic cultures. Periodic microscopic examination and chlorophyll extracts substantiated this visual observation.

Suspended Solids, Chlorophyll, and Algal Phosphorus Relationships

In addition to the routine parameters, total and volatile suspended solids were determined in the medium-biomass phases of the seventh sampling date cultures (except the two cultures used for species identification). Suspended solids, chlorophyll and ratios of the two for each seventh-run culture are presented in Table B-1. The chlorophyll/solids ratios for these thirty-five day old cultures are roughly equivalent for the two algae-containing series. Although the number of analyses does not permit statistical evaluation, ratios of .010 o.d./mg for total suspended solids and .025 o.d./mg for volatile suspended solids appear appropriate.

The small values for suspended solids in the abiotic and bacteria-only liquid phases were likely associated with sediments stirred up during culture separation. If it is assumed that equivalent amounts of sediment were included in the algae-containing liquid phases, then the proportion of sediment weight to total suspended solids is small in the algae-containing cultures. Discounting this small fraction of sediments in the seventh

TABLE B-1

SUSPENDED SOLIDS AND CHLOROPHYLL RELATIONSHIPS IN THE MEDIUM-BIOMASS PHASES OF THE SEVENTH SAMPLING PERIOD CULTURES

Culture	Total SS mg/25ml	Volatile SS mg/25ml	Chlorophyll o.d./25ml	Chlorophyll Total SS o.d./mg	Chlorophyll Volatile SS o.d./mg
I A+B	27.9	9.0	.3025	.0108	.0336
III A+B	23.4	7.4	.180	.0077	.0243
I A	13.2	6.0	.140	.0106	.0233
III A	17.0	6.5	.180	.0106	.0277
I O	1.5	.6	0	-	-
II O	1.3	.5	0	-	-
III O	1.3	.7	0	-	-
I B	1.0	.7	0	-	-
II B	1.6	.5	0	-	-
III B	.9	.5	0	-	-

sampling period liquid phase, an estimate of the phosphorus content of the algal biomass was made by dividing Total Centrifugable Solids Phosphorus per 25 ml (calculated from the mg/l values reported in Appendix C) by total suspended solids (dry weight) and volatile suspended solids (volatile weight) per 25 ml. The results were:

Culture	Phosphorus as a Percent of Dry Weight	Phosphorus as a Percent of Volatile Weight
I A+B	.17	.53
III A+B	.20	.65
I A	.26	.57
III A	.19	.49

The .49-.65 percent phosphorus as a part of volatile weight is well within the range of .3-.9 percent reported by Porcella et al. (30) for Oscillatoria grown on deep, anaerobic sediments.

In order to evaluate the relation between algal biomass (using chlorophyll as the indicator of biomass) and phosphorus, chlorophyll was correlated against total centrifugal solids phosphorus for all algae-plus-bacteria cultures (see Figure B-1) and for all algae-only cultures (see Figure B-2).

Correlation coefficients for the plots ($r = .870$ for the algae-plus-bacteria series; $r = .865$ for the algae-only series) indicated a high positive correlation between chlorophyll content and phosphorus in the algae. The slopes of the linear regression lines for the data were 4.184 o.d./mg-P for the algae-plus-bacteria series and 4.040 o.d./mg-P for the algae-only series. The similarity of the slopes indicates a close and constant relationship between algal biomass and cellular phosphorus for all algae-containing cultures.

Figure B-1 - Correlation plot for optical density of chlorophyll extracts against total phosphorus concentrations in media-biomass phases for all Algae-plus-bacteria cultures. Numbers are the sampling periods of the individual cultures.

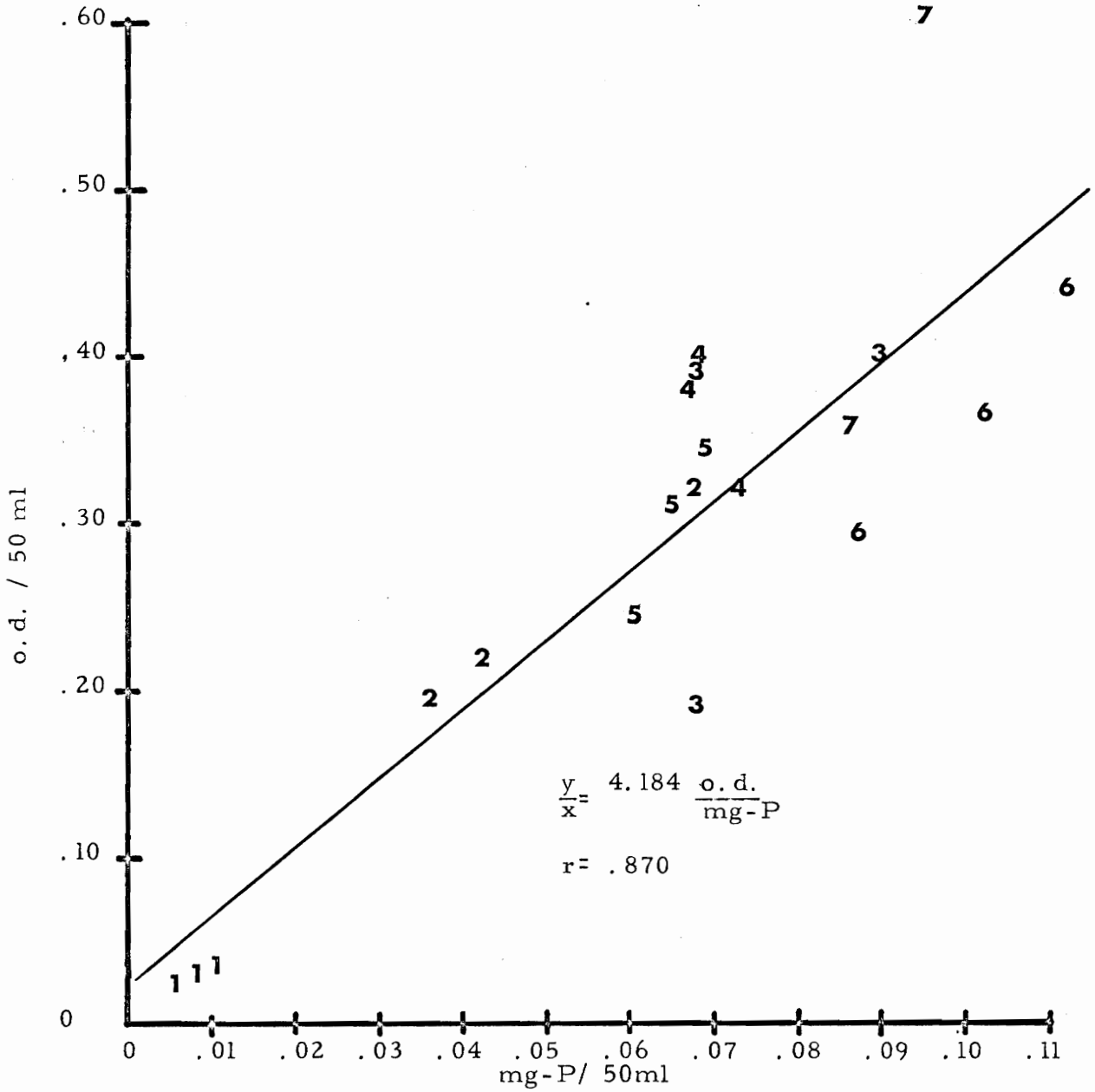
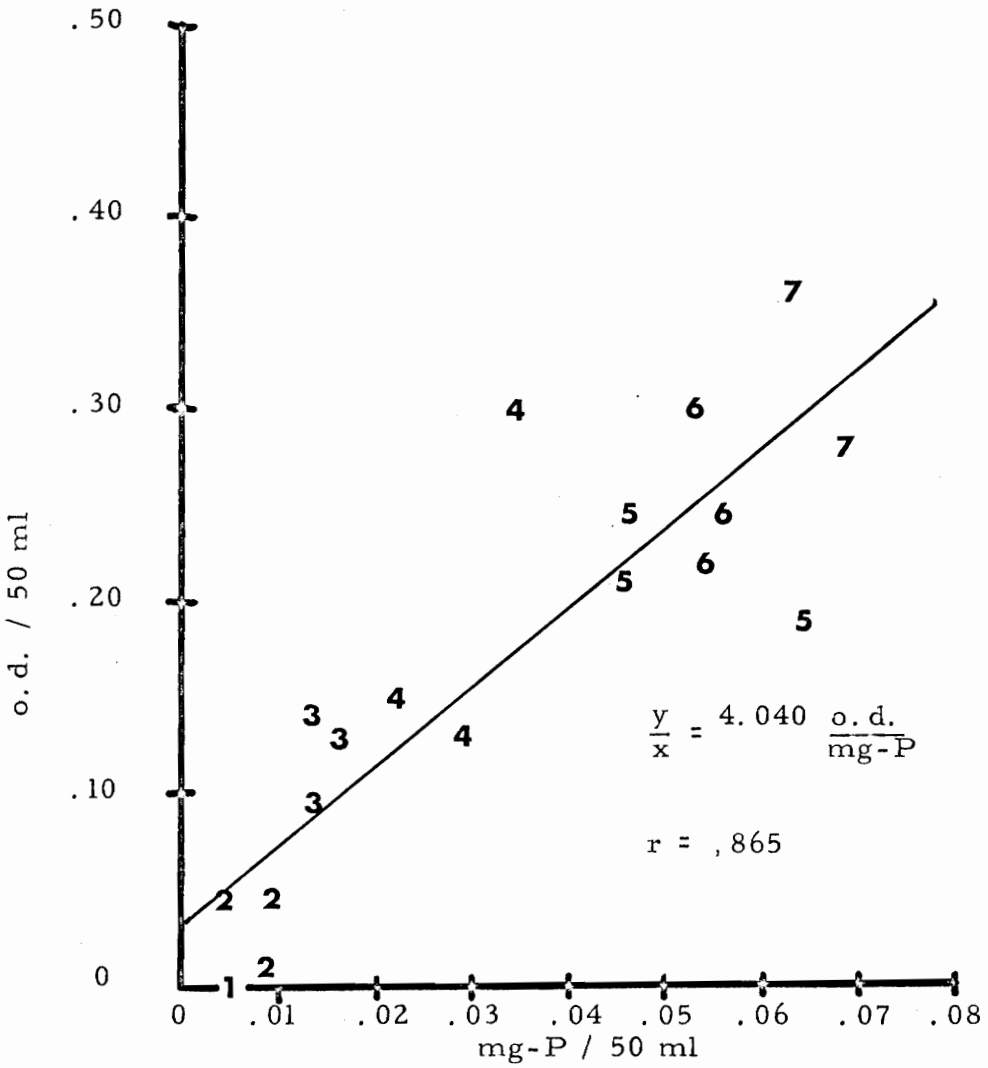


Figure B-2 - Correlation plot for optical density of chlorophyll extracts against total phosphorus concentrations in media-biomass phases for all Algae-only cultures. Numbers are the sampling periods of the individual cultures.



Non-Sediment Algal Growth Study

As a correlated experiment, algae were grown in non-sediment cultures with and without the bacterial inhibitor, potassium tellurite. Algae for inoculation of these non-sediment were taken from one of the fourth sampling period algae-plus-bacteria cultures. Freshly prepared Gorhams ASM-1 medium with phosphate was used. Chlorophyll, suspended solids and volatile solids were determined on these cultures at five and seventeen days after inoculation. The purposes of this experiment were to evaluate the growth of the blue-green algae inoculum in non-sediment cultures and to see if the potassium tellurite had the same effect in non-sediment cultures as in sediment cultures.

The results of this experiment are presented in Table B-2. The algae grew poorly in both tellurite and non-tellurite cultures. Table B-3 compares the average seventeen-day chlorophyll values for the non-sediment cultures to the fifteen-day values for sediment cultures. Fogg (55) discusses the conclusions of other investigators that there is some unknown growth factor in natural waters which is not present in defined medium. It is possible, considering the slow growth in the Gorham's medium without sediments, that some growth factor was provided by the sediments.

Of interest also is the negligible growth in the non-sediment, tellurite cultures between five and seventeen days after inoculation. Because these were not axenic cultures, this slow growth cannot be attributed to either: (a) the tellurite directly inhibiting algae growth; or (b) the tellurite inhibiting bacteria which the algae may require to produce growth factors. Whatever obstacle to growth was active

TABLE B-2

RESULTS OF THE NON-SEDIMENT
ALGAE GROWTH EXPERIMENT

	Five Days After Inoculation			Seventeen Days After Inoculation		
	Chlorophyll o.d./50ml	Suspended Solids mg/50ml	Volatile Solids mg/50ml	Chlorophyll o.d./50ml	Suspended Solids mg/50ml	Volatile Solids mg/50ml
Without Tellurite	3.25	2.0	1.2	10.5	6.0	4.8
	3.5	2.4	1.3	13.0	6.5	4.9
	4.0	2.4	1.4	13.75	6.5	5.1
	4.5	2.4	1.5	16.0	6.8	5.2
With Tellurite	1.5	E	E	2.0	2.4	1.6
	2.0	E	E	2.0	2.6	1.8
	2.0	E	E	2.5	2.8	1.8
	2.0	E	E	3.0	2.8	2.0

E - Error due to glass fiber filter adhering to weighing pan.

TABLE B-3

ALGAE GROWTH IN NON-SEDIMENT CULTURES
COMPARED TO SEDIMENT CULTURES

	Chlorophyll in Sediment Cultures (15-Day) o.d./50ml	Chlorophyll in Non-Sediment Cultures (17-Day) o.d./50ml	Non-Sediment/ Sediment Percent
Without Tellurite	163.	13.3	8%
With Tellurite	61.	2.4	4%

in the non-sediment culture may also have been responsible for the lag in algae growth in the algae-only (tellurite) sediment series.

As mentioned in Materials and Methods--Standard Curves and Estimates of Error, most of the replicate sampling done for chlorophyll was part of this non-sediment growth study. Table B-4 presents the results of a statistical analysis of the chlorophyll values obtained. Of interest is the fact that the standard error as a percent of the mean was fairly constant for all cultures.

TABLE B-4

MEANS AND STANDARD ERROR OF THE MEAN FOR CHLOROPHYLL ANALYSIS IN FOUR NON-SEDIMENT CULTURES

Culture	Optical Density		
	Mean (N = 4) o.d./50ml	Standard Error o.d./50ml	Standard Error as a Percent of the Mean %
5-Day With Tellurite	1.88	± 0.125	6.6
17-Day With Tellurite	2.38	± 0.24	10.1
5-Day Without Tellurite	3.80	± 0.248	6.5
17-Day Without Tellurite	13.31	± 1.134	8.5

APPENDIX C

ANALYTICAL DATA

The following pages present data discussed previously. Analysis names and culture designations are explained below:

A+B	- algae-plus-bacteria cultures
A	- algae-only cultures
O	- abiotic cultures
B	- bacteria cultures
I, II, III	- Roman numerals were used in conjunction with the culture designations listed above to identify all cultures and results from each culture.
Chlorophyll o.d./50ml	- Optical density of the acetone extract of 50 ml of medium-biomass mixture. Where 50 ml of the mixture would not pass a filter, the optical density of 25 ml was multiplied by 2.0.
Ortho-P mg-P/l	- Orthophosphate phosphorus in the medium-biomass phase. The concentration applies to the phase after it was diluted to 250 ml after culture separation.
Total Soluble P mg-P/l	- Total soluble phosphorus in the medium-biomass phase. The concentration applies to the phase after it was diluted to 250 ml after culture separation.
Total Centrifugable Solids P	- Total phosphorus associated with centrifugable solids in the medium-biomass phase. The concentration applies to the solids as they were dispersed in 250 ml of medium.
Total P in Medium	- Total soluble phosphorus and total centrifugable solids phosphorus concentrations (mg/l) were added together then multiplied by .25 to determine the mass (mg) of total phosphorus in the medium-biomass phase.

Percent of Culture in Medium	- For cultures in which total sediment phosphorus was analyzed, Total P in Medium was divided by Total Phosphorus in Sediment (mg/culture) plus Total P in Medium and multiplied by 100. This is an index of the amount of phosphorus transferred from the sediment to the liquid phase.
Saloid-bound P, mg/.5 g and mg/culture	- Weak-salt solution extractable sediment phosphorus. Includes only inorganic phosphorus. Reported as a concentration (mg/.5 g) of the air-dried sediments and as the total mass in the culture's sediment.
Inorganic Sediment P	- Mehta extract inorganic phosphorus. Does not include saloid-bound phosphorus. Reported as a concentration (mg/.5 g) of the air-dried sediment and as the total mass in the culture's sediment.
Total Sediment P	- Mehta extract total phosphorus. Does not include saloid-bound phosphorus. Reported as a concentration (mg/.5 g) of the air-dried sediment and as the total mass in the culture's sediment.
Error	- Where laboratory accidents resulted in an absence of data or in data that obviously is in error, results are not reported.

FIRST SAMPLING DATE

		Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/1	Total Soluble P mg-P/1	Total Centri-fugable Solids P mg-P/1	Total P in Medium mg-P	Percent of Culture P in Medium %
I	A+B	.030	28×10^7	.182	1.285	.168	.363	Error
II	A+B	.025	28×10^7	.078	.147	.112	.065	
III	A+B	.035	25×10^7	.128	.213	.214	.107	
I	A	0	8×10^6	.160	.241	.100	.085	
II	A		----------Not Sampled----------					
III	A		----------Not Sampled----------					
I	O	0	40×10^3	.098	.160	.083	.061	
II	O		8×10^5	.115	.193	.096	.072	
III	O		37×10^3	.100	.136	.125	.065	
I	B	0	36×10^7	.045	.206	.110	.079	1.24
II	B		50×10^7	.037	.155	.079	.048	
III	B		30×10^7	.049	.208	.094	.075	

FIRST SAMPLING DATE--Continued

	Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
	mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I A+B	.0052	.040	.527	4.075	.857	6.626	3.866
II A+B	.0068	.054					3.974
III A+B	.0080	.070					4.366
I A		.051	.428		.687		Error
II A							
III A							
I O		.069	.540		.719		Error
II O	.0076	.062					4.052
III O	.0079	.068					4.290
I B	.0083	.073	.482	4.244	.714	6.286	4.402
II B	.0066	.050					3.816
III B	.0052	.040					3.856

SECOND SAMPLING DATE

		Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/l	Total Soluble P mg-P/l	Total Centri-fugable Solids P mg-P/l	Total P in Medium mg-P	Percent of Culture P in Medium %
I	A+B	.195	6×10^9	.099	.200	.725	.231	3.94
II	A+B	.220	4×10^9	.019	.221	.857	.270	
III	A+B	.320	$< 1 \times 10^9$	.063	.126	1.357	.371	
I	A	.045	2×10^7	.029	.208	.190	.099	1.41
II	A	.045	2×10^7	.103	.178	.087	.066	
III	A	.010	8×10^7	.096	.179	.178	.089	
I	0	0	8×10^7	.116	.212	.079	.073	1.35
II	0		10×10^7	.062	.129	.091	.055	
III	0		4×10^7	.071	.107	.062	.042	
I	B	0	Error	.027	.247	.339	.147	2.13
II	B		1×10^9	.043	.170	.142	.078	
III	B		Error	.051	.163	.786	.237	

SECOND SAMPLING DATE--Continued

		Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
		mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I	A+B	.00052	.004	.482	3.703	.732	5.625	3.842
II	A+B	.00078	.006					3.854
III	A+B	.00114	.008					3.513
I	A	.00140	.012	.504	4.333	.808	6.947	4.299
II	A	.00114	.009					3.943
III	A	.00200	.014					3.485
I	O	.00090	.007	.487	3.797	.683	5.325	3.898
II	O	.00092	.007					3.790
III	O	.00077	.007					4.562
I	B	.00045	.004	.500	4.389	.768	6.741	4.389
II	B	.00056	.005					4.445
III	B	.00058	.005					4.315

THIRD SAMPLING DATE

		Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/l	Total Soluble P mg-P/l	Total Centri-fugable Solids P mg-P/l	Total P in Medium mg-P	Percent of Culture P in Medium %
I	A+B	.400	16×10^7	.056	.128	1.803	.482	8.98
II	A+B	.395	7×10^7	.012	.076	1.364	.360	6.38
III	A+B	.190	7×10^7	.018	.055	1.364	.355	6.61
I	A	.140	5×10^7	.019	.138	.281	.105	2.00
II	A	.095	2×10^7	.010	.160	.277	.109	2.07
III	A	.130	4×10^7	.014	.126	.330	.114	2.06
I	O	0	18×10^5	.081	.140	.074	.054	
II	O		7×10^5	.083	.199	.081	.070	
III	O		$< 1 \times 10^5$	.114	.145	.063	.052	
I	B	0	1×10^9	.098	.232	.170	.100	1.72
II	B		2×10^9	.083	.293	.217	.128	
III	B		1×10^9	.055	.247	.163	.103	

THIRD SAMPLING DATE--Continued

		Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
		mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I	A+B	.00159	.012	.473	3.570	.647	4.883	3.774
II	A+B	.00177	.014	.482	3.808	.669	5.285	3.950
III	A+B	.00147	.012	.455	3.702	.616	5.012	4.068
I	A	.00306	.024	.464	3.639	.656	5.144	3.921
II	A	.00112	.009	.464	3.720	.643	5.155	4.009
III	A	.00120	.010	.446	3.702	.652	5.413	4.151
I	O	.00075	.008					5.328
II	O	.00081	.008					4.326
III	O	.00078	.006					3.829
I	B	.00081	.007	.473	4.074	.665	5.728	4.307
II	B	.00055	.005					4.518
III	B	.00117	.011					4.698

FOURTH SAMPLING DATE

		Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/l	Total Soluble P mg-P/l	Total Centri-fugable Solids P mg-P/l	Total P in Medium mg-P	Percent of Culture P in Medium %
I	A+B	.320	12×10^7	.069	.312	1.464	.444	7.46
II	A+B	.400	13×10^7	.055	.513	1.357	.468	
III	A+B	.390	22×10^7	.023	.293	1.339	.408	
I	A	.250	18×10^7	.029	.278	.446	.181	3.06
II	A	.300	46×10^7	.019	.108	.696	.201	
III	A	.130	4×10^7	.024	.358	.589	.237	
I	O		$< 1 \times 10^6$	.064	.345	.165	.128	2.04
II	O		9×10^6	.062	.279	.209	.122	
III	O		5×10^6	.068	.321	.205	.132	
I	B		55×10^8	.060	.375	.185	.140	2.37
II	B		46×10^8	.072	.335	.094	.130	
III	B		37×10^8	.065	.611	.137	.187	

FOURTH SAMPLING DATE--Continued

		Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
		mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I	A+B	.00084	.007	.451	3.733	.665	5.505	4.139
II	A+B	.00153	.012					3.904
III	A+B	.00080	.006					3.748
I	A	.00105	.008	.482	3.639	.759	5.730	3.775
II	A	.00176	.014					3.976
III	A	.00131	.010					3.816
I	O	.00102	.008	.487	3.797	.790	6.159	3.898
II	O	.00139	.011					3.958
III	O	.00093	.008					4.322
I	B	.00069	.006	.487	4.199	.670	5.777	4.311
II	B	.00093	.009					4.851
III	B	.00077	.007					4.553

FIFTH SAMPLING DATE

	Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/l	Total Soluble P mg-P/l	Total Centri-fugable Solids P mg-P/l	Total P in Medium mg-P	Percent of Culture P in Medium %
I A+B	.310	11×10^8	.028	.152	1.304	.364	5.92
II A+B	.245	13×10^8	.016	.116	1.214	.333	
III A+B	.345	15×10^8	.020	.131	1.375	.377	
I A	.210	3×10^8	.020	.160	.918	.270	5.01
II A	.245	54×10^8	.028	.211	.929	.285	
III A	.190	1×10^8	.012	.112	1.286	.350	
I O		1×10^6	.049	.152	.100	.063	0.81
II O		41×10^6	.070	.238	.105	.086	
III O		$< 1 \times 10^6$	.078	.159	.162	.080	
I B		36×10^8	.041	.245	.126	.093	1.64
II B		37×10^8	.122	.250	.241	.123	
III B		20×10^8	.065	.254	.162	.104	

FIFTH SAMPLING DATE--Continued

		Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
		mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I	A+B	.00119	.009	.473	3.584	.763	5.782	3.789
II	A+B	.00151	.012					3.977
III	A+B	.00137	.011					4.024
I	A	.00234	.019	.424	3.447	.629	5.114	4.065
II	A	.00180	.015					4.164
III	A	.00172	.015					4.357
I	O	.00099	.009	.460	4.166	.857	7.761	4.528
II	O	.00137	.011					4.004
III	O	.00112	.009					4.013
I	B	.00108	.009	.468	3.884	.674	5.594	4.150
II	B	.00104	.009					4.313
III	B	.00093	.008					4.315

SIXTH SAMPLING DATE

		Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/l	Total Soluble P mg-P/l	Total Centri-fugable Solids P mg-P/l	Total P in Medium mg-P	Percent of Culture P in Medium %
I	A+B	.440	11×10^8	.025	.223	2.250	.618	9.93
II	A+B	.365	43×10^8	.029	.214	2.053	.567	10.40
III	A+B	.295	16×10^8	.028	.536	1.750	.572	9.16
I	A	.300	50×10^8	.031	.214	1.078	.323	6.26
II	A	.220	17×10^8	.033	.257	1.096	.338	5.68
III	A	.245	21×10^8	.051	.245	1.125	.343	6.17
I	O		12×10^6	.068	.268	.132	.100	1.59
II	O		32×10^6	.050	.428	.129	.139	2.07
III	O		25×10^6	.036	.178	.337	.129	2.64
I	B		111×10^8	.116	.315	.268	.146	2.44
II	B		36×10^8	.097	.378	.217	.149	2.50
III	B		60×10^8	.069	.366	.238	.151	2.61

SIXTH SAMPLING DATE--Continued

		Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
		mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I	A+B	.00134	.011	.460	3.776	.683	5.606	4.104
II	A+B	.00172	.012	.464	3.234	.701	4.886	3.485
III	A+B	.00119	.010	.491	4.109	.678	5.674	4.184
I	A	.00237	.018	.460	3.485	.638	4.833	3.788
II	A	.00278	.021	.473	3.566	.745	5.617	3.770
III	A	.00223	.018	.446	3.596	.647	5.216	4.031
I	O	.00115	.010	.487	4.235	.710	6.174	4.348
II	O	.00143	.012	.495	4.141	.786	6.576	4.183
III	O	.00127	.011	.492	4.258	.549	4.751	4.327
I	B	.00128	.011	.482	4.115	.683	5.831	4.269
II	B	.00130	.011	.491	4.122	.692	5.810	4.198
III	B		Error	.491	4.137	.670	5.645	4.213

SEVENTH SAMPLING PERIOD

		Chloro-phyll o.d./50ml	Bacteria Per ml Sediment Wash	Ortho-P mg-P/l	Total Soluble P mg-P/l	Total Centri-fugable Solids P mg-P/l	Total P in Medium mg-P	Percent of Culture P in Medium %
I	A+B	.605	39×10^8	.031	.397	1.911	.577	
II	A+B		Sample Used for Algae Identification					
III	A+B	.360	40×10^8	.023	.223	1.928	.538	
I	A	.280	18×10^8	.025	.281	1.382	.416	
II	A		Sample Used for Algae Identification					
III	A	.360	16×10^8	.012	.195	1.268	.366	
I	O		32×10^6	.027	.312	.482	.199	
II	O		64×10^6	.076	.553	.110	.166	
III	O		40×10^6	.045	.160	.134	.074	
I	B		60×10^8	.075	.314	.326	.160	
II	B		50×10^8	.095	.285	.265	.138	
III	B		26×10^8	.092	.366	.464	.208	

SEVENTH SAMPLING PERIOD--Continued

		Saloid-Bound P		Inorganic Sediment P		Total Sediment P		Sediment Weight
		mg/.5 g	mg/culture	mg/.5 g	mg/culture	mg/.5 g	mg/culture	grams
I	A+B		.008					
II	A+B							
III	A+B		.002					
I	A		.002					
II	A							
III	A		.003					
I	O		.000					
II	O		.000					
III	O		.000					
I	B		.001					
II	B		.000					
III	B		.000					

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VITA

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After receiving a Bachelor of Arts degree from the University of Virginia, Charlottesville, in 1967 with a major in Biology, he was forcefully employed by the U.S. Department of the Army as a military public health specialist. Serving his country at Fort Gordon, Georgia and in Southeast Asia, he kept his nose clean but still managed to catch the biggest amoeba Southeast Asia had to offer.

After military service, he was employed by the Prince William County Public Health Department from 1969 to 1972. Here he learned all about septic tanks and local politicians.

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Gerald O. Peters, Jr.

MICROBIOLOGICAL INFLUENCES ON PHOSPHORUS RELEASE FROM AEROBIC LAKE SEDIMENTS

by

Gerald Owen Peters, Jr.

(ABSTRACT)

The role of sediments in regulating nutrient availability in lakes and reservoirs has been the subject of many recent studies. Classical theories concerning the regulation of phosphorus release from sediments by oxidation-reduction potential neglect the potential of microorganisms to transfer large amounts of phosphorus out of sediments.

Aerobic surface sediments collected from the eutrophic Occoquan Reservoir, Virginia, were treated with ethylene oxide to reduce the populations of living organisms. These sediments served as the sole source of phosphorus in cultures inoculated with bacteria and/or blue-green algae collected from the Occoquan Reservoir. Inoculum composition was maintained for the thirty-five day incubation period with selective inhibitors. An abiotic culture series served as a control. Cultures were harvested at five-day intervals and were analyzed for phosphorus fractions in sediment and medium, chlorophyll, bacteria in the sediments, and air dry weight of the sediments.

Short term (less than five days) release of total and inorganic phosphorus was roughly equal for all cultures. After five days growth of algae correlated with rapid transfer of phosphorus from the sediments.

A large proportion of the released phosphorus was incorporated into the algae. Cultures containing just bacteria showed only slightly higher phosphorus release rates than abiotic cultures.

Blue-green algae appear to act as a sink for phosphorus, permanently upsetting the phosphorus equilibrium between sediment and water. The sediments continuously release phosphorus to restore the equilibrium. Bacteria appear to have little effect beyond the mineralization of relatively small quantities of organic phosphorus.