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A STUDY OF PARTICULATE ORGANIC ^{32}PHOSPHORUS

IN A SIMULATED SEDIMENT-WATER SYSTEM

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T O J U D Y

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CHAPTER I

INTRODUCTION

For at least the past 25 to 30 years, the role of phosphorus in lake eutrophication and organism growth has been extensively investigated. During this period, sophisticated research tools became available. With the aid of radioactive elements, e.g., ^{14}C and ^{32}P, and of better analytical techniques, the many processes involved in lake eutrophication could be investigated much more quantitatively.

The processes involved in lake eutrophication have regained the interest of many scientists and engineers because of the recognition that the urbanization of a lake's watershed greatly increases the rate of nutrient influx to the lake. As early as 36 years ago, this problem was emphasized by Hasler (1947).

The predominant nutrients, phosphorus and nitrogen, found in wastewater and agricultural runoff can greatly accelerate lake eutrophication. Since it is technologically feasible and economical to remove phosphorus from wastewater, phosphorus removal seems to be the logical choice for solution to the problem. However, phosphorus

present in the soil, and hence available through leaching and runoff to freshwater and saltwater environments, could be more difficult to regulate.

The available information in the literature indicates that sediments serve as a sink for phosphorus with the net flux of phosphorus from water to the sediments. However, if the phosphorus present in wastewater and agricultural runoff were controlled prior to entering a lake, would the sediments of that lake act as a phosphorus buffer to the overlying water?

Mortimer (1942) studied the exchange of dissolved substances between mud and lake water in Lake Esthwaite and Lake Windermere of the English Lake District. He suggested that, if the reducing power of the mud were increased sufficiently to result in reduction of the mud surface, productivity would increase since the supply of solutes to the water could augment plankton production. Hutchinson and Bowen (1950) using radiophosphorus detected the movement of phosphorus from the mud into the hypolimnion in Linsley Pond. Harter (1968) working with eutrophic lake sediments demonstrated that the sediments act as a phosphorus buffer to keep a relatively constant phosphorus level in the overlying water.

It is generally agreed upon in the literature that sediments affect the nutrient status of lake water. The mechanisms involved have not been clearly defined. Indeed, the role of the sediment is still little understood.

This investigation of the sediment-water interface was directed toward clarifying the role of the heterotrophic community of sediment

in the phosphorus exchange processes between sediment and water. Specifically, the study dealt with the biodegradation and subsequent release of particulate organic phosphorus from the sediment-water interface to the overlying water. To do this, it was necessary to design and to use a continuous-flow apparatus which would simulate the sediment-water interface of a lake. This approach was therefore a feasibility study as well. That is, the apparatus had to be evaluated for its usefulness in studying sediment-water interactions in addition to the degradation of a phosphorus form. Also, a realistic form of phosphorus had to be used. Particulate organic phosphorus was considered to be the most prevalent phosphorus form settling to the sediment in a lake. The other phosphorus forms would probably be mineralized in the hypolimnion, recycled, and would never settle to the sediment. Therefore, algal cultures were grown, and their particulate organic phosphorus components were labelled with ^{32}P. By so doing, this approach was more of a modelling attempt than a kinetic study.

During February, June, and August of 1972, a total of twenty sediment cores representing the top 10 cm of sediment were taken from one sampling site approximately 500 meters perpendicular to the middle of the dam on Lake Thunderbird. Each core was subdivided into two 5-cm sections. The concentration and dominant genera of bacteria were analyzed. Fungi were cultured but not identified in this study.

Algal cultures were cultured with varying quantities of phosphorus to determine the culturing technique necessary for optimum ^{32}P labelling of the particulate organic phosphorus fraction of the

cells. The algal culture was then extracted according to Fitzgerald and Nelson (1966) to isolate the particulate organic ^{32}P components.

Aliquots of the labelled particulate organic ^{32}P components were placed in eight columns of the sediment-water apparatus at the mud-water interface. By flowing water through the apparatus and monitoring the effluent for ^{32}P, iron, manganese, sulfate, electrode potential, and pH, it was possible to follow the response of the biological community to changes in certain environmental control factors. The ^{32}P release from the sediment as a result of mineralization, etc., could then be investigated as a function of these changes occurring in the overlying water.

The results obtained from this investigation verified the important role that the sediment heterotrophic community has in the interchange of nutrients from the sediment to the overlying water during summer stratification when the hypolimnion is anaerobic.

The degradation of particulate organic ^{32}P was shown to occur at a rate which was much slower than that previously suggested in the literature. It was also indicated that the movements of particulate organic phosphorus from the sediment to the hypolimnion during (simulated) summer stratification was dependent mostly on the mineralization processes. The capacity of the sediment to sorb phosphate and the redox condition also affected the interchange of phosphorus to the overlying water.

The feasibility of the sediment-water apparatus to study sediment-water interactions was quite justified based on the responses of the system to changes in the overlying water.

CHAPTER II

LITERATURE SURVEY

Sources of Phosphorus in Lakes

Questions usually raised concerning investigations of nutrient cycling include the source of the nutrient, the form in which the nutrient occurs throughout the cycle, the manner of transformation of the nutrient, and the rate at which the nutrient is exchanged and recirculated.

Goldschmidt (1937) estimated that on the average, for each square centimeter of the earth's surface, 160 kg of igneous rocks containing approximately 0.16 kg of phosphorus have been eroded since the earth's origin. Via weathering of solid rocks into smaller and smaller aggregates, the gradually formed loose particulates may eventually enter into solution or become colloidal or particulate suspensions in natural waters (Spear, 1970).

Lake nutrient budget studies by Frink (1967) indicated that the sediments of an eutrophic lake are greatly enriched in phosphorus. Mackereth (1966) and Wentz and Lee (1969) suggested several sources of the nutrient phosphorus within lake sediments: (1) sedimentation

process in combination with autochthonous organic matter, (2) erosion of phosphorus-containing minerals located in the watershed and consequent deposition in an unaltered form, (3) coprecipitation with iron and manganese, (4) sedimentation in combination with allochthonous organic matter, (5) sorption, and (6) association with carbonates.

Autochthonous may be defined as being formed in the lake itself by life processes or physical-chemical processes (Ruttner, 1953). Spear (1970) suggested three types of autochthonously formed phosphate bearing sediments: (1) those derived from sedimentation of biologically fixed particulates including plant and animal remains, (2) those derived from chemical precipitation occurring within the boundaries of the lake, and (3) those derived from sorption reactions.

Allochthonous may be defined as having been introduced from outside the lake (Ruttner, 1953). The organic component of allochthonous sediments should not be disregarded. Ruttner (1953) reported that microscopic examination of these sediments revealed measurable quantities of plant tissues and, to a lesser extent, animal remains.

Phosphorus Cycling in Lakes

The phosphorus cycle has been investigated in part by several researchers (Hutchinson and Bowen, 1947 and 1950; Armstrong and Harvey, 1951; Rigler, 1956, 1961, 1964a, and 1964b; Watt and Hayes, 1963; and, Rigler and Chamberlain, 1968a and 1968b). Wentz (1967) summarized possible interactions of phosphorus within a lake basin (Figure 1).

Disregarding allochthonous phosphorus input, phosphorus is said

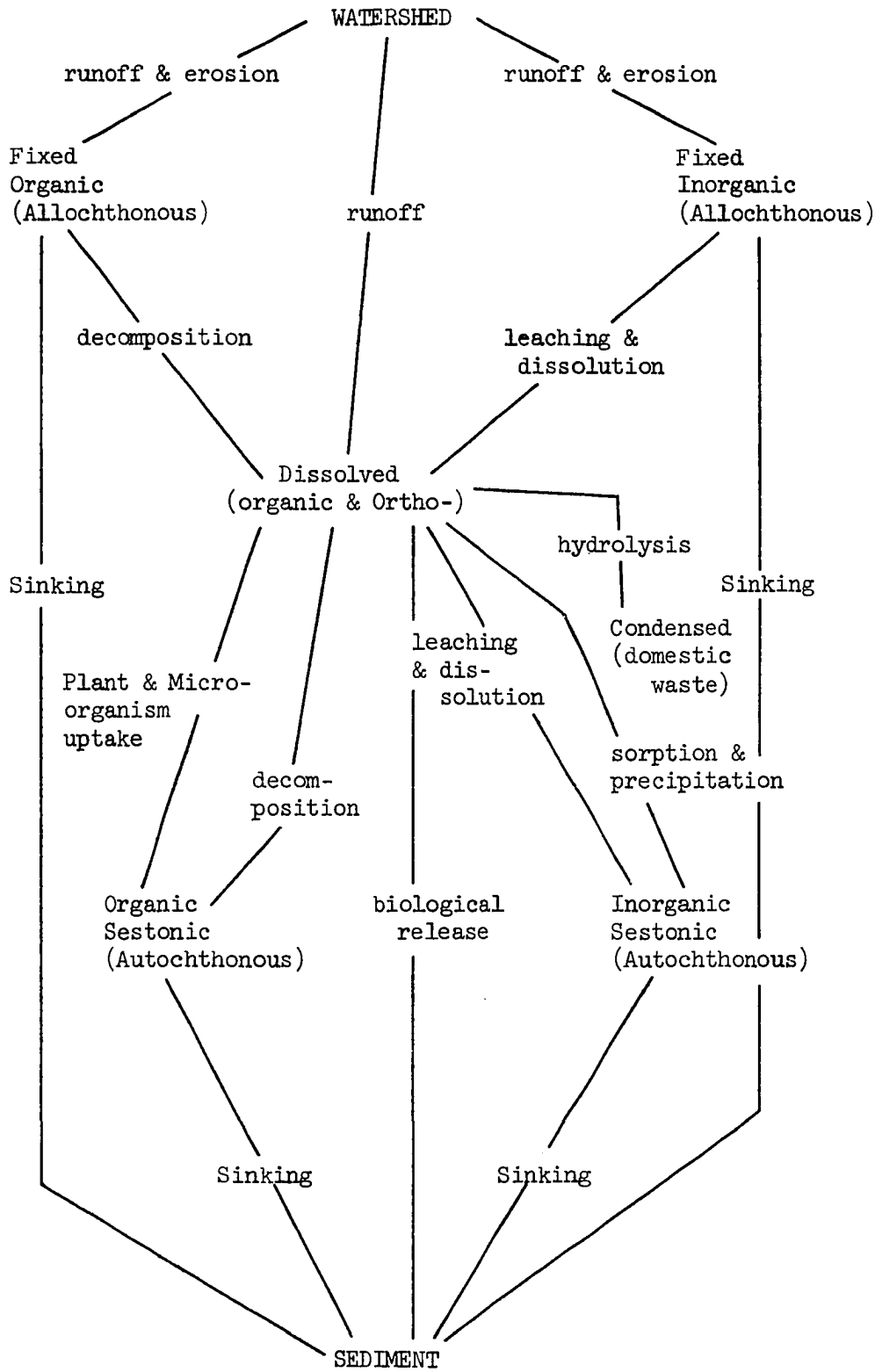


Figure 1. Phosphorus Cycle in a Lake System

to be released into the epilimnion from the littoral region as a result of decaying aquatic plants. The phytoplankton in the open epilimnion tend to assimilate some of the released phosphorus and store most of the remaining. Soluble inorganic and organic phosphorus compounds are secreted. Following this, there is a degeneration (or hydrolysis) to the ionic (or ortho) form by microorganisms such as bacteria, actinomycetes, and fungi in the water.

Finally, the phytoplankton and higher plant and animal remains settle to the sediment in the lake. During this time, those easily biodegradable phosphorus components, eg., orthophosphate, sugar phosphate, are released or decomposed prior to reaching the sediment-water interface. Most of the remaining organic phosphorus material is further degraded by the heterotrophic microorganisms in the sediment.

Hayes and Phillips (1958) represented the dynamic relationship of phosphorus and sediments as:

P in aqueous phase ⟶ P in solid phase

(Small fraction of total) (Large fraction of total)

Particulate phosphorus was shown to be the predominate phosphorus form in the sediment.

Figure 2 represents a proposed phosphorus model based on the models by Berger and Heath (1968) for particulate matter entering marine sediments and by Keeney (1973) on nitrogen cycling in sediments. The historical (unmixed) sediments are overlaid with an active sediment zone which is constantly being affected by physical and biological processes. This layer of sediment is generally regarded as

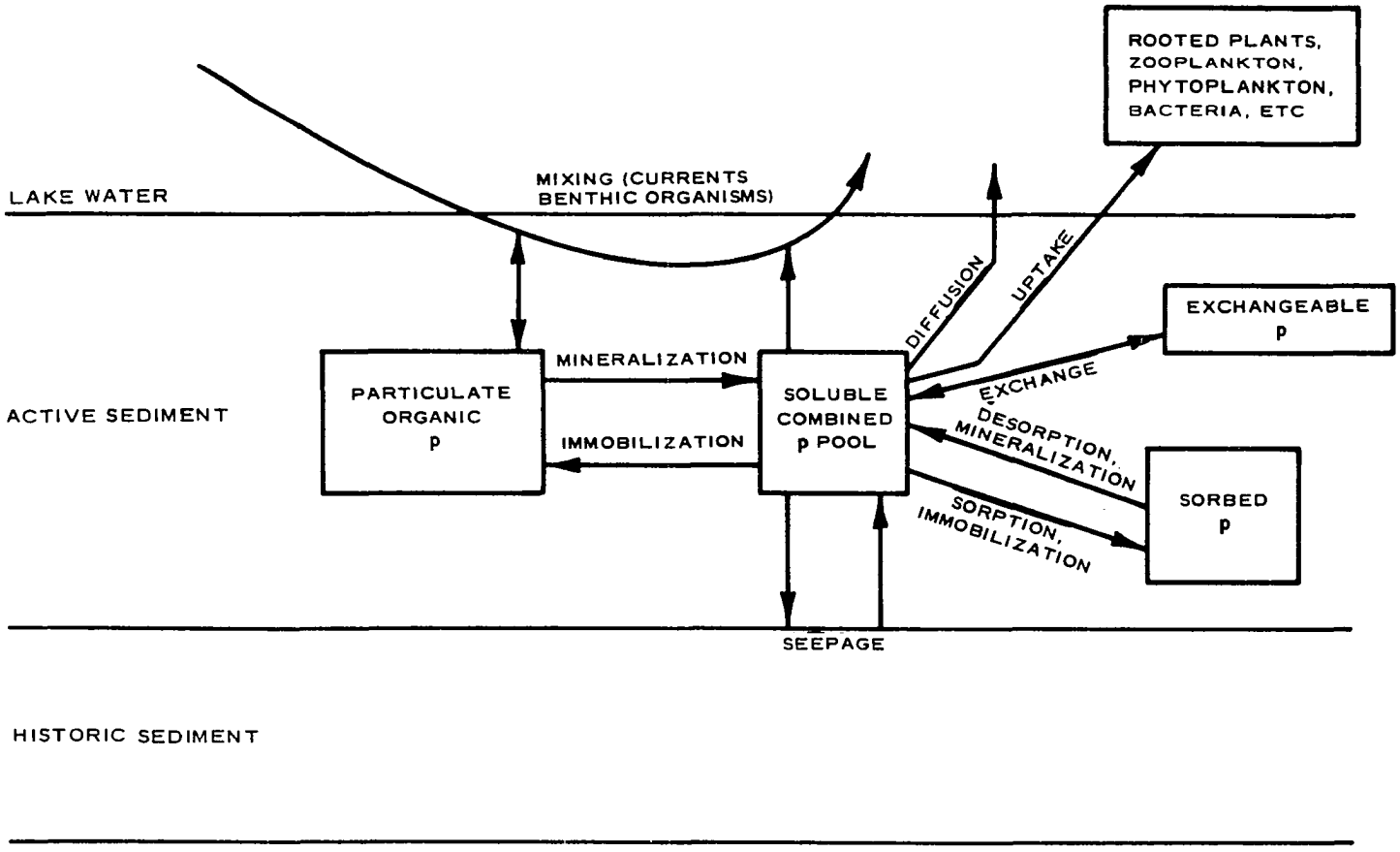


Figure 2. Sediment-Water Interchange Model for Phosphorus

being anywhere from a few centimeters thick to possibly 20 cm. This active sediment layer remains relatively constant in thickness. Depending on the rate of sedimentation, the deposition process builds up the historical layer of sediment.

With the use of radiophosphorus, ^{32}P, many investigators have studied the exchange of phosphorus in the lake ecosystem. Coffin et al. (1949) added 100 millicuries ^{32}P to a stratified acid-bog lake. At the end of 8 weeks, the radiophosphorus was only barely detected in the mud. Similarly, Rigler (1956) added radiophosphorus to the surface water of a small acid-bog lake. He recorded a 77 percent loss of radiophosphorus from the water and plankton in 4 weeks, but only 2 percent was lost through the outlet of the lake and 3 percent was lost to the mud. He concluded that there was a turnover of "mobile" phosphorus in the epilimnion with phosphorus of the littoral organisms. With respect to this exchange, the turnover time of phosphorus of the epilimnion was found to be 3.5 days. He later postulated that the turnover of radiophosphorus in lakes under natural conditions was primarily caused by bacteria. However, the investigation by Hayes and Phillips (1958) using ^{32}P showed that approximately 4 percent of the planctonic cells that utilize phosphate settle out to the sediment each day.

Pomeroy (1960) used radiophosphorus in studying the residence time of dissolved phosphate in natural waters. His studies showed that a dynamic equilibrium exists between phosphate dissolved in the water and phosphorus in the plankton, benthic organisms, bacteria, sediments, and dissolved organic materials. He suggested that the

turnover rate of phosphate was more important than the phosphate concentration in maintaining highly productive systems. Also, Phillips (1964) concluded that there is a continual exchange of phosphorus between water and solids within natural waters and that the quantitative distribution of phosphorus between the phases represents a state of dynamic equilibrium.

Similarly, Pomeroy et al. (1972) studied the flow of phosphorus in several shallow estuaries on the Georgia coast. It was indicated that release of phosphorus from the sediments was primarily biologically mediated; direct equilibrium between sediments and overlying water was of secondary importance.

These investigators have stated or implied that the phosphorus concentration in the overlying lake water is mediated to an extent by the heterotrophic assemblage of organisms in the sediment as well as in the overlying water. However, the main difference between their investigations was that the exchange rates of phosphorus in the aquatic ecosystem varied from one biological system to another. Hayes et al. (1952) using ^{32}P in Nova Scotia lakes found the turnover time for phosphorus in water to be 5.4 days for Bluff Lake, 7.6 days for Punch Bowl Lake, and 17 days for Crecy Lake, and the turnover time for phosphorus in solids to be 39 days for Bluff Lake, 37 days for Punch Bowl Lake, and 176 days for Crecy Lake. Watt and Hayes (1963) also showed this to be true. They indicated that exchange rates vary seasonally within the same system.

Other investigators have stated that the phosphate concentration in waters overlying sediments is buffered by solubility and adsorption

equilibria at the sediment-water interface (Stumm and Morgan, 1970). Chemical interactions of phosphate with Fe(III), Al(III), clays, and Ca(II) were suggested as relevant mechanisms.

In summary, the net flow of phosphorus in a lake is generally to the sediments as a consequence of continual deposition of organic matter. The systems governing the release of sediment phosphorus back to the phosphorus cycle in the overlying water include chemical, physical, and biological interactions.

Chemical Systems Dominating Phosphorus in Lake Sediments

The anionic exchange capacity in soils was extensively studied by Toth (1937), Dean and Rubins (1947), Dalton et al. (1952), Hannapel et al. (1964), Hsu (1965), Kafkafi et al. (1967), Tandon and Kurtz (1968), Juo and Ellis (1968), Syers and Walker (1969a and 1969b), Blanchar and Hossner (1969), Harter (1969), Syers et al. (1969), and Mahapatra and Patrick (1969). Several types of anionic exchanges involving clay minerals were postulated: (1) replacement of hydroxyl ions as shown by Dickman and Bray (1940) and McAuliffe et al. (1947), (2) adsorption of phosphate and other anions, which have size and geometry similar to those of the silica tetrahedron, by fitting onto the edges of the silica tetrahedron sheets and growing as extensions of these sheets (Grim, 1953), (3) exchange sites due to unbalanced charges within the lattice, e.g., an excess of aluminum in the octahedral positions (Schofield, 1940 and 1949).

In lake sediments, two chemical systems dominate the phosphate ion. Golterman (1967) and Golterman et al. (1969) suggested the Fe-S

phosphate system and the $Ca-CO_3$ phosphate system. Both of these systems are influenced by pH; however, only the Fe-S phosphate system is influenced by the redox conditions.

If any free iron, aluminum, or alkaline earths are present to react with the anions such as phosphate, it would be difficult to separate the effects due to such reactions from those which may be due to reactions with the clay minerals. These types of anionic exchanges are also expected in lake studies.

Fe-S Phosphate System

Einsele (1936, 1937) recognized the importance of iron in the control of phosphate ions in lake sediments. Although Einsele used pure chemical systems and laboratory studies only, valuable insight was gained. The reactions involving ferric hydroxide implied that precipitation and sorption reactions were utilized for phosphate control in sediments. Under oxidizing conditions, $FePO_4$ precipitated followed by the reduction of Fe(III) to Fe(II). Increasing the pH of the solutions to greater than 6 resulted in less phosphate being sorbed onto the gel complex.

Laboratory and field studies on water samples were conducted by Mortimer (1941, 1942). He found that a thin film of oxidized material, a ferric hydroxophosphate complex, was present at the sediment-water interface during times when the hypolimnion contained oxygen. The depth of this layer ranged from a few millimeters to several centimeters. This layer acted as a barrier to prevent phosphate from being released into the overlying water. When the oxygen

was depleted from the hypolimnion, this oxidized layer became non-existent, and the release of phosphate from the sediment to the water occurred. That is, ferric iron was reduced to ferrous, and sulfate was reduced to sulfide via bacterial action (Golterman, 1967). The reduced iron and sulfide precipitated, thus releasing phosphate ion into solution. These reactions are represented as follows (Stumm and Morgan, 1970):

$$FePO_{4(s)} + e^- \rightleftharpoons Fe^{2+} + PO_4^{3-} \quad (1)$$

$$OH^+ + SO_4^{=} + 8e^- \rightleftharpoons 4H_2O + H_2S \quad (2)$$

$$H_2S \rightleftharpoons H^+ + HS^- \rightleftharpoons 2H^+ + S^{=} \quad (3)$$

$$Fe^{=} + S^{=} \rightleftharpoons FeS_{(s)} \quad (4)$$

The ferric-hydroxy-phosphate system is pH dependent. There is a competition between the hydroxide ion and phosphate for the ferric ion, with the hydroxide ion favored at the higher pH range while at or below a pH of 5.3 ferric phosphate is formed.

Stumm and Morgan (1970) suggested that in the neutral and slightly alkaline pH range the precipitate is probably a metastable ferric compound containing both phosphate and hydroxide in variable proportions, depending upon the pH.

According to Stumm and Morgan (1970), the heterogeneous equilibria, characterized by the solubility of $AlPO_4$ (variscite), $FePO_4$ (strengite), and $Ca_{10}(PO_4)_6(OH)_2$ (hydroxyapatite) and the adsorption of phosphate on clays, determine the distribution between the aqueous

phase and the solid phase in the sediments. In contradiction to this was the work of Williams et al. (1971). They studied the native inorganic phosphorus levels in calcareous and noncalcareous Wisconsin lake sediments and concluded that the phosphorus concentrations were largely determined by the amount of extractable iron derived from an amorphous iron complex. They did not find variscite or strengite that is said to contain the major form of aluminum- and iron-bound phosphorus in the sediments. In fact, they stated that discrete aluminum and iron phosphates have not been recorded in unfertilized soils or in lake sediments. Shukla et al. (1971) and Delfino et al. (1969) arrived at similar conclusions. However, Schofield (1968) studied the mechanism of phosphate fixation in organic sediments in four oligotrophic lakes of the Adirondack Mountain range of New York. Through a sequential extraction of the sediments, he concluded that iron phosphates and occluded forms were dominant in oxidized sediment and aluminum phosphates were prevalent in reduced sediments. The relative stability of the aluminum phosphates was greater than iron phosphates under reducing conditions. He went on to state that there was a high concentration of organic phosphorus in the sediment.

$Ca\text{-}CO_3$ Phosphate System

Spear (1970) suggested that the $Ca\text{-}CO_3$ phosphate system is most important in hard water lakes. Degens (1965) stated four requirements for phosphate deposition in lakes: (1) a pH greater than 7, (2) the presence of a calcareous substance and a system that is calcium saturated with respect to its carbonate content, (3) phosphate

concentrations exceeding 0.1 mg P/l, and (4) a nondepositional environment, i.e., where organic matter is entirely decomposed and recycled in the lake water.

Soluble phosphate appears not to be controlled by apatite, $CaHPO_4$, in natural water where some calcium is present. The solubility of hydroxyapatite, $Ca_5OH(PO_4)_{3(s)}$, is less than that of $CaHPO_{4(s)}$. Stumm and Morgan (1970) described the solubility of hydroxyapatite as:

$$Ca_5OH(PO_4)_{3(s)} \rightleftharpoons 5Ca^{2+} + 3PO_4^{3-} + OH^-$$

$$Ca_5OH(PO_4)_{3(s)} + 3H_2O \rightleftharpoons 2(Ca_2HPO_4(OH)_2)_{surface} + Ca^{2+} + HPO_4^{2-}$$

$$(Ca_2HPO_4(OH)_2)_{surface} \longrightarrow 2Ca^{2+} + HPO_4^{2-} + 2OH^-$$

The surface complex is said to be in metastable equilibrium with the aqueous solution. Thus, Stumm and Morgan (1970) concluded that hydroxyapatite, as a stable solid phase in natural water, tends to buffer the total phosphate concentrations in these waters.

Gerhold and Thompson (1969) reported that calcium hydroxyapatite, $Ca_5OH(PO_4)_3$, was the principal phosphate mineral present in samples of bottom sediments from Western Lake Erie. The majority of the phosphate in the samples studied by Gerhold and Thompson was amorphous, not mineral phosphate. They found that, under optimum conditions, the phosphate released from calcium hydroxyapatite was sufficient to keep the algae at a maximum growth rate.

In summary, the soil scientists during the 1950's pioneered the phosphorus extraction procedures as applied to soils and found that

inorganic phosphorus will occur as iron-, aluminum-, and/or calcium-bound forms in soils. Likewise, in sediments the same forms are expected to be predominant. In calcareous sediments, the calcium-bound phosphorus form is expected; whereas in noncalcareous sediments, iron- and aluminum-bound phosphorus forms are expected. The iron- and aluminum-bound phosphorus may be either adsorbed or hydrated amorphous iron and aluminum oxides or occluded as a sesquioxide coating on silicate minerals. The latter forms are generally not found in a reduced sediment environment. Calcium-bound phosphorus forms are predominantly found as discrete calcium phosphate (apatite) or as hydroxyapatite (Chang and Jackson, 1958).

Physical Factors Affecting Sediment-Water Interaction

Lee (1970), in his review of the factors affecting exchange reactions between sediment and water, suggested that the hydrodynamics of a lake system are the rate-controlling step in exchange reactions. He suggested that currents transport leached materials, e.g. phosphates, away from the sediment, allowing concentration-dependent exchange reactions to proceed toward establishing equilibrium. Furthermore, mixing in the waters would hinder settling of particles and would thereby enhance the exchange reactions. He suggested that mixing would carry unleached particles to the sediment-water interface where exchange reactions take place more readily. Finally, mixing was suggested as the agent responsible for bringing sediment interstitial water to the surface where it could mix with the overlying water.

The morphometry and geographical location of a freshwater lake are very important when evaluating the physically induced mixing of water and sediment. If the fetch of a lake is great, more turbulent wave action will persist for longer periods of time. Thus, the shore material will erode faster and increase the amount of suspended matter already in the body of water. Seasonal temperature, day length, and watershed mineral composition greatly affect the mixing action as well as sediment composition. Temperature not only affects the viscosity of lake water, but it also may affect the degree of mixing in a lake. The water in the watershed area will enter the lake and spread horizontally along the thermogradient in the lake most closely associated with that of the incoming water. Depending on the season of the year and geographical location, the mixing capability of the lake currents can be important in the exchange of materials between sediments and water. Long growing seasons will enhance the organic buildup of lake water, further affecting sediment composition and quantity. Similarly, the mineral composition and size of the watershed area can affect the amount of deposition of allochthonous material into the water as well as the settling characteristics of the minerals in the lake.

The depth of sediments actively involved in exchange reactions has been generally agreed to be on the order of 5 to 20 cm (Davis, 1968; Delfino et al., 1969; Hynes and Greib, 1970; and Veith and Lee, 1971). Borthleson (1970) studied the distribution of phosphorus in several different lakes in northern and central Wisconsin. Where the influx of phosphorus had sufficiently increased, the mixed

deposition of phosphorus in the sediments extended to a depth of 5 to 10 cm. This suggests an active mixing or exchange reaction zone much deeper than Mortimer (1941) or Hayes et al. (1952) suggested.

Nauman (1930) was first to propose that the sediments in natural waters could be separated into an upper zone and a lower historical layer where essentially no mixing or exchange reactions occur. The upper layer or active deposition zone is the layer from which exchange reactions take place. He suggested that the depth of this layer was about 10 cm for lakes in Europe and 5 to 10 cm for the continental shelf. Lee (1970) reported that recent studies in the University of Wisconsin Water Chemistry Laboratory supported the concept of a mixing zone extending down to about 5 to 10 cm below the sediment-water interface for many lakes in which the sediment-water interface was well defined.

The amount of mixing occurring in sediments was suggested by Lee (1970) to be one of the most important aspects of exchange reactions. Rittenberg et al. (1955) showed that interstitial water may contain about 50 times more soluble orthophosphate than the overlying water. This would suggest an important role of mixing in determining the rate of phosphorus exchange.

Mortimer (1941, 1942) suggested that exchange reactions are dependent on molecular diffusion in sediments. The early investigators did not consider the role of mixing in sediment-water interchange. Hayes et al. (1952) stated that only a superficial layer 1 cm thick was involved in the exchange of phosphorus between sediment and water.

According to Stumm and Morgan (1970), the movement of dissolved molecules in water is on the order of 10^{-5} cm^2/sec. This rate was based on the fact that most chemical reactions in natural waters take place at phase discontinuities, and interstitial waters present a variety of solid solution interfaces (Stumm and Morgan, 1970) in which sorption and exchange reactions may occur. Sharma (1970) stated that because of hindrance, diffusion of ions through even a few centimeters in sediments may take years. However, several investigators (Stumm and Leckie, 1971; Hynes and Greib, 1970) implied that diffusion processes may be significant in sediment-water interchanges. Diffusion is probably more important in deep sections of lakes during stratification.

The oxidized microzone on the surface of sediments was suggested by Mortimer (1942) as being caused by the diffusion of oxygen into the upper layers of sediments, and as a result the oxidation of the reduced elements such as Fe(II) to Fe(III) occurred. However, the more popular view represented by Gorham (1958) was that turbulent mixing of the surface sediment into the overlying water resulted in the oxidation of Fe(II) to Fe(III) and sedimentation of $Fe(OH)_3$ onto the sediment surface.

Organisms can also mix the sediments and aid in the exchange processes. Whitten and Goodnight (1967) studied the role of tubificids in the transfer of ^{32}P in an aquatic ecosystem. They suggested that tubificids in conjunction with bacteria aid in recycling radiophosphorus from detritus and sediments. Anaerobic fermentation reactions of bacteria in the sediments could cause bubbles of carbon

dioxide and methane gas to rise through the sediments and water resulting in sediment-water mixing.

Biological Influence on the Phosphorus Cycle

According to Alexander (1971), the tissues of aquatic plants and animals, the corresponding land forms, water-soluble inorganic phosphate, and the insoluble aluminum, iron, and calcium phosphates of land and sediments are the main substrates of concern in the three major kinds of reactions by which the phosphorus cycle is regulated. These three reactions are: (1) heterotrophic mineralization, by bacteria and fungi, to orthophosphate, (2) immobilization of inorganic phosphorus by the proliferation of photoautotrophs and heterotrophs resulting in a decrease of available phosphorus, and (3) solubilization of insoluble inorganic phosphates. Solubilization is effected through the production of organic, nitric, or sulfuric acids which, for example, solubilize the phosphorus in apatite, or by the production of H_2S, which may dissolve the phosphorus in ferric phosphates of bottom sediments. The rate of the solubilization process could limit primary production if the ambient phosphorus readily available to the photoautotrophs is low. This could in turn affect the higher herbivores which feed on this food source, and ultimately all carnivores would be affected in the system.

Harris (1957) used ^{32}P and the organisms Artemia and Gammarus and found an initial conversion of radioactive inorganic phosphate into organic phosphorus followed by incorporation into the experimental crustaceans. No ^{32}P was incorporated into the crustaceans when

antibiotics were added, leading to the conclusion that phosphate was incorporated into microorganisms before ingestion by the higher herbivores and carnivores. Hoffman (1956) followed dissolved organic phosphorus and phosphate in plankton. He concluded that there were no available estimates of the quantity of phosphate in nature that was a result of autolytic remineralization or a result of bacterial decomposition. Rodina (1963) found that detritus consisted of a great variety of microscopic biotopes for bacteria, which differ from one another in their structure and chemical characteristics. These findings further suggested an important role for the bacteria in recycling phosphorus.

Heterotrophic organisms which mineralize phosphorus are ubiquitous and numerable. According to Alexander (1971), the mineralization of most phosphorus-containing constituents of microbial and plant cells is rapid, except when they are adsorbed onto inanimate materials or complexed in such a way that increases the resistance of the molecule to enzymatic breakdown. This type of protection is considered to be common in soil and sediments.

Phosphorus in the biosphere participates in high-energy metabolic intermediates (Lear, 1970). Consequently, it is generally found as the orthophosphate esterified to an organic compound as a monoester, diester, or triester. Polyphosphates have been found in microorganisms and certain algae (Table 1). The current hypothesis as to the metabolic significance of the polyphosphates (known as volutins) is that these are storage forms for the ready availability of phosphate to the cell metabolic pathway.

Organic phosphorus compounds such as ribose-1-phosphate,

TABLE 1

ALGAE IN WHICH POLYPHOSPHATES HAVE BEEN FOUND

Cyanophyta	
Anabaena variabilis	*O. amoeua*
Cylindrospearmum licheniforme	*O. limnosa*
Gloeothece sp.	*Phormidium ambiguum*
Lyngbya aerugineo-coerulea	*P. frigidum*
L. amplivaginata	*P. unicinatum*
Oscillatoria sp.	
Chlorophyta	
Acetabularia mediterranea	*Hydrodictyon recticulatum*
Chlorella sp.	*Mougeotia sp.*
C. ellipsoidea	*Oedogonium sp.*
C. pyrenoidosa	*Rhopalocystis eleifera*
C. vulgaris	*Scenedesmus sp.*
Cladophora sp.	*Spirogyra sp.*
Cosmarium sp.	*Ulothrix sp.*
Enteromorpha sp.	*Zygnema sp.*
Bacillariophyta	Xanthophyta
Fragilaria sp.	*Vaucheria sp.*
Navicula sp.	
Cryptophyta	Euglenophyta
Chilomonas sp.	*Euglena gracilis*
Rhodophyta	Charophyta
Ceramium sp	*Chara sp.*

creatine phosphate, and acetyl phosphate are unstable and hydrolyze into an organic moiety and inorganic phosphate. Those forms known to be active in metabolism, such as glucose-6-phosphate, and glycerol-1-phosphate, are resistant to hydrolysis (White et al., 1964). Hence, certain organophosphorus compounds tend to be quite stable in aquatic environments in that they are insoluble in water.

The phospholipids are the building blocks of the cellular membranes. This group of particulate organic phosphorus compounds is probably the most prevalent phosphorus component of dead and decaying plant and animal detritus at the sediment-water interface. Most of the other major cell constituents, i.e., proteins, amino acids, and sugar phosphates, would be degraded by heterotrophic microorganisms in the overlying water as the plant or animal tissue slowly settled to the sediment-water interface.

This study is particularly concerned with the regeneration of the phosphorus which is bound in the cell membranes. This is typically the particulate organic phosphorus group referred to in lake sediments. The mineralization of this component by the sediment heterotrophic community is little understood.

Table 2, which is taken from Lear (1970), shows the phosphorus distribution in several types of organisms. It can be concluded that the phosphorus distribution in plants and animals is quite variable with little associated patterns. Benson and Shibuya (1962) stated that for Scenedesmus obliquus and Chlorella pyrenoidea cells grown at moderate light intensities, phosphatidyl glycerol was the most abundant organic phosphorus compound.

TABLE 2

PHOSPHORUS DISTRIBUTION IN ORGANISMS*

Organism	Inorganic P	TCA** Soluble	Phospho-lipid	Nucleic Acid	Phospho-protein
Tribolium confusum, flour beetle	13.44	28.6	29.0	25.6	2.1
Tetrahymena, protozoan					
Mating type I		7.6	1.9	11.7	51.7
Mating type II		19.5	10.8	21.6	53.1
Thiobacillus, bacterium	12.3	69		18.7	
				RNA + DNA	
Serratia marcessens, bacterium		17.76	6.03	62.70 + 1.85	
		17.12	7.36	60.72 + 8.10	
		22.43	7.44	55.90 + 2.82	
		16.61	12.09	57.75 + 8.06	
Escherichia coli, bacterium	9.44	8.24	6.09	66.23 + 9.48	0.5

* Values in the table are percent of total phosphorus.
** TCA = trichloroacetic acid.

Methods Available for Sediment-Water Studies

There have been very few attempts to study specific components of a larger ecosystem. Instead, attempts have been made to construct laboratory models of ecosystems with the hope of compartmentalizing the various components of the ecosystem. This enabled studies of nutrient cycling and the association of the movement of the nutrient with the various ecosystem components. For example, Whittaker (1961) studied the movement of ^{32}P into different components of an aquarium ecosystem. However, he was not able to describe what form of ^{32}P actually moved into and out of his designated ecosystem components. This is a very important question to be considered. The form and rate of regeneration are of major concern when discussing phosphorus concentration and its effect on the biotic components of an ecosystem.

The size of laboratory models of aquatic ecosystems has typically ranged from microcosms, which include flask cultures and aquariums, to larger physical models such as an estuarine ecosystem complete with watershed and tidal fluctuations (McIntire 1969). Several other researchers resorted to the microcosm approach (Odum and Hoskin 1957; McConnell 1962; Copeland 1965) to study growth responses of algae and bacteria under different nutrient or environmental conditions.

Laboratory microcosms have enabled bioassay techniques to provide very useful information. Cairns et al. (1972) used fish in small microcosms to study their response to metals. This kind of information may be of some significance in developing bioindicators to warn of toxic metal concentrations in wastewater from industries. Cummins

et al. (1972) used leaf leachate to study the role of producers, consumers, and microbial consumers during organic enrichment. Gloyna et al. (1971) used radioisotope tracers in microcosms to study the movement of trace metals through sediments and plants.

Although microcosms provide a laboratory method for studying ecosystems, the main criticism associated with microcosms is that they are not very stable for long periods of time. Population density and community structure are severely restrained by nutrient depletion, container effects, etc. (Kubitschek, 1970). Kubitschek (1970) stated that batch culturing, which is typical of most of the microcosm attempts, results in varying cell size, composition, and functional characteristics.

McIntire (1969) emphasized the need for a shift from culture flasks to ecological investigations in nature or in simulated natural environments. He further stated that laboratory models of ecosystems would be helpful if designed properly. His work considered productivity and bioenergetics of benthic algal communities. By controlling some of the abiotic components such as light, carbon dioxide, dissolved oxygen, temperature, and current velocity, he hoped to relate the organism responses.

Gahler (1969) studied nutrient interchange at the sediment-water interface. Through field and laboratory investigations some comparisons could be made. Four polyethylene pools were placed in the lake being investigated. Two of the pools had bottoms, and two were exposed to the sediment. Over 7 months, chemical and biological observations were made on the water trapped in these pools. Comparisons

were made of lake water quality in each of these pools. For the laboratory investigation, lake sediment was placed in the bottom of 6-inch-diameter, 6-foot-high glass columns. Lake water was placed in the columns and monitored for chemical changes that could be attributed to the sediment. He maintained aerobic and anaerobic conditions in the overlying water.

Mackenthun (1968) used ^{32}P in an aquarium microcosm in a manner similar to that of Gahler. He found that the amount of phosphorus released to the overlying water was small. In fact, if ^{32}P were placed only 0.6 cm below the mud surface, it was essentially lost to the system. Phosphorus in solution was increased when he circulated the overlying water with air bubbles.

All of these studies involving batch culturing may be affected by some of the problems suggested by Kubitschek (1970).

There is an increasing need for the development of larger physical models of ecosystems which attempt to more closely identify physically and operationally with the ecosystem. These would retain similar attributes of the smaller microcosms as previously described and also would be more stable with respect to complexity, i.e., biotic communities and populations. The more realistic physical models would enable most of the dynamic processes that exist in nature to be simulated in the laboratory. According to Holm-Hansen (1969), the nutrients being supplied to a natural system are continually being replenished; therefore, a method of nutrient removal and nutrient replenishment must be provided. This dynamic process, as well as water supply and other associated elements that are typical of an ecosystem,

must be considered when building the physical model.

As stated earlier, there is a need for the development of techniques and models which consider the interrelations of the ecosystem components. The literature is quite devoid of investigations which attempt to answer the following questions: how are nutrients exchanged from one ecosystem component to another; in what chemical or physical form are the nutrients being exchanged; are some forms of nutrients more readily available than other forms; and, is there a need and a way to control the form in which the nutrients are being exchanged in the ecosystem?

Continuous culture techniques were suggested by Kubitschek (1970) as offering the appropriate controlled conditions for ecological studies. He stated that even though very few attempts at continuous culturing have been made to date, the development of these techniques in the study of community growth kinetics should offer great promise.

Macura et al. (1965) applied similar techniques to study the decomposition of uniformly tagged glucose in soil with different inorganic nitrogen and phosphorus levels. Their investigation included the percolation of ^{14}C-labelled glucose through soil columns with different inorganic nitrogen and phosphorus levels. The percolation of this solution was at a continuous flow. They found that the amount of glucose-carbon mineralized to carbon dioxide was higher if nitrogen and phosphorus were added together with the glucose. The quantitative relationship between the assimilation and oxidation of glucose-carbon depended on the nitrogen and phosphorus concentration. It was in

inverse proportion to the mineral element level. Also, the rate of glucose oxidation was strongly influenced by the flow rate. Through this continuous-flow method, a dynamic steady state was maintained for a long period of time.

Macura and Kunc (1965) used the same method to study the biological immobilization of mineral forms of nitrogen and phosphorus. Through the use of this method, they were able to differentiate between physical-chemical adsorption of mineral elements and biological immobilization over a given period of time. They concluded that biological immobilization of nitrogen and phosphorus was closely associated with glucose decomposition. Also, a correlation was found between the immobilization of nitrate nitrogen and carbon dioxide evolution during glucose decomposition to the quantity of glucose utilized. The ratio of the amount of glucose-carbon assimilated by the soil microflora to the amount of nitrogen immobilized depended on the C:N ratio in the added glucose solution. Finally, they stated that the use of soil columns of different weights and heights made it possible to determine phosphorus retention capacity in different layers of the soil columns.

Basic to the continuous culture technique is that individual cells are maintained in as uniform an environment as feasible. This realistically requires a completely mixed cultivation chamber. Macura et al. (1965) and Macura and Kunc (1965) did not meet this requirement. The action of percolation of a liquid through a saturated soil column could result in preferred paths of flow through the column and might present many different microenvironments for

biological and chemical reactions to occur. However, it represents a more realistic environment in which to study soil microbial decomposition under saturation conditions.

Consequently, this investigation of a sediment and water system considers a modification of the continuous flow, batch culture, and chemostat methodology as a means to study biological degradation processes. This study is far more qualitative than quantitative, for it attempts to evaluate a more realistic continuous flow technique to study sediment-water interactions. Specifically, the study is of the biodegradation of particulate organic phosphorus and the subsequent release of particulate organic phosphorus from the sediment-water interface to the overlying water. The approach is more of a modelling attempt than a kinetic study.

CHAPTER III

EXPERIMENTAL PROCEDURE AND METHODS

General

The sediment and lake water used in this study were taken from Lake Thunderbird, Cleveland County, Norman, Oklahoma. One sampling site was used as the source of all sediment samples. Delfino et al. (1969) showed that the sediment phosphorus concentration increases with increasing depth of overlying water. Anderson (1970) stated that the extremely fine clays (<1.0 μ) were dominant in this region. For these reasons, it was decided that the sampling station would be located in the deep profundal region of the lake. The deepest region of the impoundment was located about 500 meters perpendicular to the middle of the dam where Little River and Hog Creek channels combine. This sampling site was located below 55 feet of overlying water.

Sediment Biota Characterization

For this study, it was found necessary to develop a technique whereby the sediment could be adequately sampled, and procedures by which the bacterial concentration, the identification of genus, and the organic carbon concentration could be determined from the same samples. A vertical characterization of the sediment was desired, so a core sampler was designed in which the sediment samples could be

acquired. The same core sampler used to acquire sediment cores for the above analyses will be used as part of the continuous-flow apparatus.

During February, June, and August of 1972, a total of 20 sediment cores representing the top 10 cm of sediment were taken. Each core was subdivided into two 5-cm sections. For each of these sections, the bacterial concentration was determined, and as many of the genera of heterotrophic bacteria as possible were identified. The fungi were noted but not classified. Macroorganisms were not found in enough quantity to warrant further investigation. Next, each of the subsamples of sediment was oven-dried at 110°C for 1 hour, cooled, and weighed. Organic carbon was determined by weight difference after the oven-dried sample was ignited at 600°C for 1 hour, cooled, and weighed.

Several mixed algal culturing techniques were attempted to determine the best method for labeling the organic components of the algae with ^{32}P. The algae were cultured under constant lighting using four 40-w G.E. cool-white fluorescent lamps and a 20°C incubation temperature. A modified KNOP's algal media was selected for culturing the algae. The ^{32}P, as $KH_2{}^{32}PO_4$, was added to the culture, and the cell weight as well as the aqueous ^{32}P was monitored for the next few days. When most of the ^{32}P was particulate and not in the aqueous phase, the algae were extracted according to Fitzgerald and Nelson (1966). Several other extraction procedures were also attempted in order to identify the particulate organic ^{32}P compounds in the algae.

Following the extraction of the particulate organic ^{32}P component in the algae, the residue was then placed in each column of the

sediment-water apparatus at the sediment-water interface. Approximately 15 to 20 mg of the residue was added.

The day before the algae was extracted, eight sediment cores were obtained from the sampling site on Lake Thunderbird. These cores were kept in the refrigerator at 4°C until they were inserted into the continuous flow apparatus.

The same day that the particulate organic ^{32}P was extracted, the sediment cores were extruded until the top 10 cm were remaining in the tube. The tubes were then ready to be placed in the continuous-flow apparatus. All lake water and distilled water used in the experimental runs were autoclaved for 1 hour at 121°C and 18 psi in 4-ℓ pyrex reservoirs.

Run 1

The mineralization study of the particulate organic ^{32}P in the first run consisted of eight sediment-water systems containing eight sediment cores (Figure 3). Columns 1-4 were operated with a continuous flow of filtered, sterile Lake Thunderbird water passing over the sediment. The heterotrophic community in the sediment of columns 1 and 2 was alive. The sediment in columns 3 and 4 had been autoclaved for 20 minutes at 121°C and 18 psi in the polycarbonate tubes prior to setting them up in the continuous-flow apparatus. These columns were maintained in the sterile condition. Columns 5-8 were operated with a continuous flow of filtered, sterile distilled water passing over the sediment. The heterotrophic community in the sediment of columns 5 and 6 was alive. The sediment cores in columns 7 and 8 were sterilized in the same manner as columns 3 and 4.

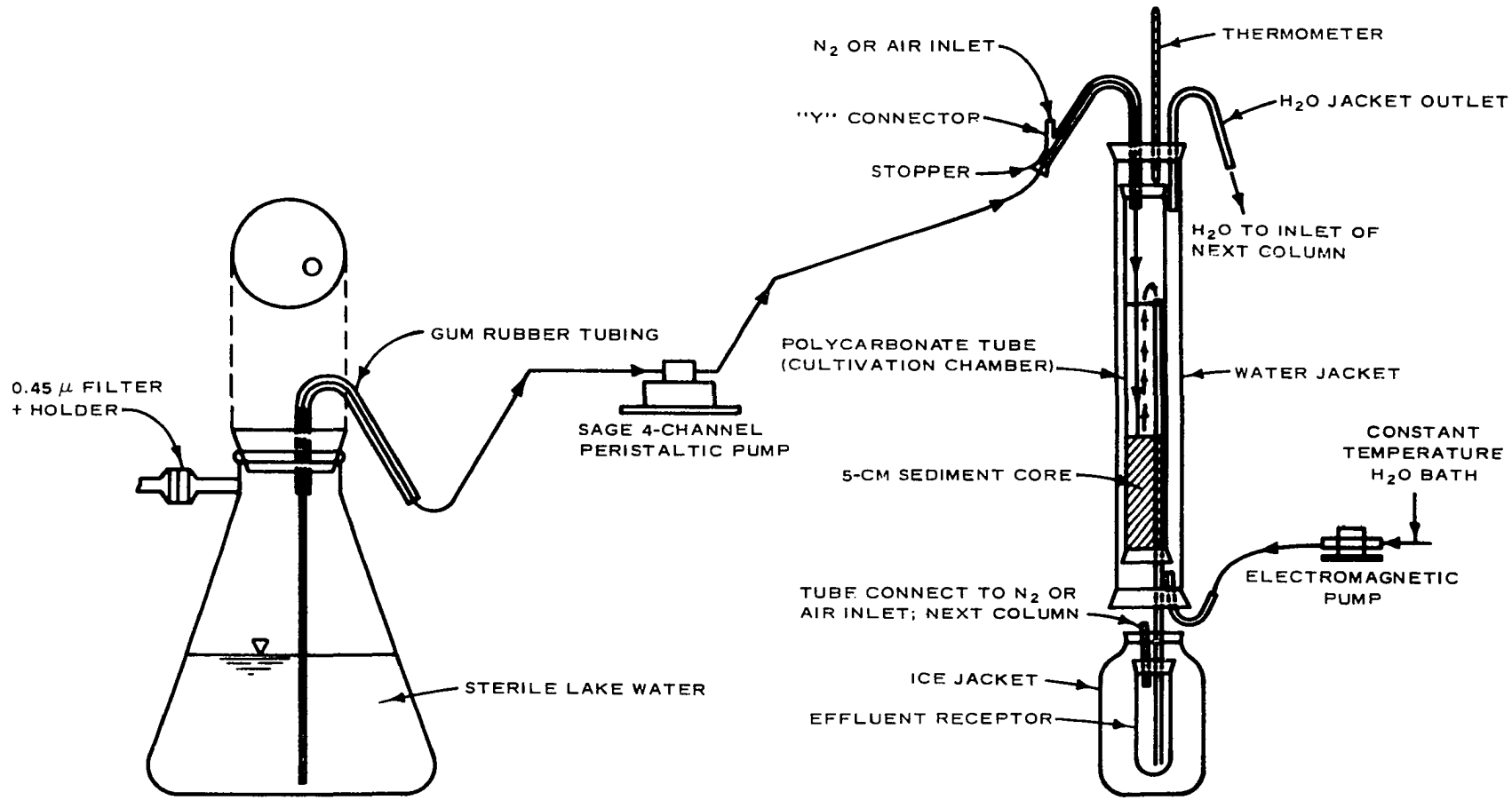


Figure 3. A Sediment-Water, Continuous-Flow Apparatus

The operating temperature for this system was kept constant at 18 ± 1°C using a recirculating, constant-temperature water bath. This temperature represents the maximum summer temperature of the hypolimnion immediately above the sediment in Lake Thunderbird.

At a constant flow rate of 2 ml per hour, the hydraulic retention time (HRT) in each column was approximately 24 hours. The total volume of water overlying the sediment was approximately 50 ml.

The effluent from each column was collected in 60-ml glass tubes sitting in ice in thermal containers. The effluent was analyzed every 24 hours for ^{32}P, pH, redox potential, iron, manganese, and sulfate.

On day 29, a few milliliters of formalin were added to columns 3 and 4. On day 37, nitrogen gas was purged through column 1 for the duration of the run. On day 38, nitrogen gas was purged through column 6 for the duration of the run. The duration of this run was 59 days for columns 1 and 2, 52 days for columns 3 and 4, and 46 days for columns 5 through 8.

Run 2

Columns 1 and 2 were continued from the prior run, and six more columns were set up in the apparatus. The specific activity of ^{32}P in the algae was increased because of low counting rates in the prior run. Each of the eight cores was purged with filtered, compressed nitrogen gas in order to better evaluate the redox condition in the overflow water without reaeration.

Columns 1, 3, 5, and 7 were operated with a continuous flow of filtered, sterile Lake Thunderbird water passing over the sediment.

Columns 2, 4, 6, and 8 were operated in a similar manner except that an additional 0.5 mg P/ℓ was added as KH_2PO_4 was added to the lake water. The same temperature (18 ± 1°C) used in the last run was also used in this run.

The HRT's were varied for this run. Columns 1-5 had an HRT of about 24 hours with a total water volume of approximately 50 ml over the sediment. Columns 5 and 6 had an HRT of 12 hours with 50 ml of water over the sediment. Columns 7 and 8 had an HRT of 6 hours with 25 ml of water over the sediment.

The same parameters were analyzed as in the last run. These included ^{32}P, pH, redox potential, iron, manganese, and sulfate.

The duration of this run was 45 days. After this run was completed, attempts were made to determine where and in what form the remaining ^{32}P was in the sediment columns.

Sediment Sampling

In order to sample the sediment in Lake Thunderbird according to the requirements of this research, a core sampler was designed (Appendix A). The core sampler was 28 inches long and was weighted at the top end with 25 lb of steel. This section of the core, which comprised the widest part of the sampler (approximately 4 inches), was threaded to allow for the attachment of a cable from a winch.

For each sampling trip, at least five polycarbonate tubes and the tip of the core sampler were autoclaved for 15 minutes at 121°C and 18 psi with number 4 rubber stoppers inserted in each end of the polycarbonate tubes. These tubes were then taken to the sampling

site. On each sampling date, 5 sediment cores were taken for bacterial analysis. For each of the 5 sediment samples, a sterile polycarbonate tube was inserted into the core sampler. The core sampler was allowed to "free-fall" through the water and drive itself into the sediment. After bringing the sampler up with the winch, the polycarbonate tube containing between 15 to 20 cm of sediment was carefully removed, and the water overlying the sediment was decanted off.

The sediment was extruded by blowing in the opposite end of the polycarbonate tube until only the top 10 cm remained in the tube. This 10-cm section was extruded in two equal 5-cm sections into sterile, 50-ml polycarbonate centrifuge tubes. These tubes were then stoppered and placed on ice until they could be taken back to the laboratory for analysis.

Four sampling trips on January 25, June 7, June 20, and August 16, 1972, were made so that the minimum (5°C) and maximum (18°C) temperature of the lake water immediately above the sediment could be determined. The hypolimnion was found to contain: 11 mg/ℓ dissolved oxygen on January 25, 1972; <1.0 mg/ℓ dissolved oxygen on June 7, 1972; and 0.0 mg/ℓ dissolved oxygen on June 20, 1972, and August 16, 1972. Hence, the sediment was obtained under aerobic and anaerobic hypolimnion conditions.

Three 8-inch-diameter Ekman dredge samples of sediment were also taken on the above dates and analyzed for macroorganisms. Only one chironomidae larva was found in all of the samples taken.

Isolation of Heterotrophic Microorganisms

Each of the 5-cm sediment subsamples was carefully washed with 125 ml of sterile, distilled water into a sterile 250-ml Erlenmeyer flask. A presterilized, 1-inch magnetic stirring bar was added to each flask. The contents were vigorously stirred for 15 minutes. An aliquot from each flask was then diluted. Six 1-ml samples from the final dilution were filtered through 0.45-μ Millipore filters. These filters were then incubated in 47-mm plastic petri dishes. Each dish contained an absorbent pad impregnated with 2 ml of sodium caseinate broth (Appendix B).

Three of the plates were incubated aerobically in the dark at 25 ± 1°C. The other three plates were incubated in the dark at 25 ± 1°C using two BBL GASPAC anaerobic chambers. The aerobic plates were incubated for 18 to 24 hours, while the anaerobic plates were incubated for 48 to 72 hours.

The initial isolation of colonies was very distinct. This media was used with equal success as a broth or agar. Through several transfers of colonies from broth to agar or vice versa, this media seemed quite capable of supporting high bacteria colony counts and distinctive colony morphology.

Bacteria Concentration

Each of the 5-cm sediment subsamples was carefully washed with 125 ml of sterile, distilled water into a sterile 250-ml Erlenmeyer flask. The contents of this flask were stirred for 15 minutes with a magnetic stirring bar. The following was the dilution followed for

the first sampling run: A sterile, 10-ml pipette was inserted into the 250-ml flask (while still mixing), and 10 ml of sample was pulled out and deposited into 90 ml of sterile, distilled water contained in a dilution bottle. After shaking the dilution bottle vigorously for 1 minute, 10 ml was removed and again deposited into a dilution bottle containing 90 ml of sterile, distilled water.

5 cm sediment $\longrightarrow$ 125 ml $\xrightarrow{10\ ml}$ 90 ml H_2O $\xrightarrow{10\ ml}$ 90 ml H_2O

For the next three sampling runs, the following dilution scheme was followed: A sterile, 10-ml pipette was inserted into the 250-ml flask containing the suspended sediment, and 10 ml of sample was removed and placed into a dilution bottle containing 90 ml of sterile, distilled water. After shaking vigorously for 1 minute, 1 ml was removed with a 1-ml sterile pipette and placed in a dilution bottle containing 99 ml of sterile, distilled water.

5 cm sediment $\longrightarrow$ 125 ml H_2O $\xrightarrow{10\ ml}$ 90 ml H_2O $\xrightarrow{1\ ml}$ 99 ml H_2O

From the last dilution bottle, each of six 1-ml aliquots was filtered through a 0.45-μ Millipore filter. Three plates were incubated aerobically for colony isolation, and three plates were incubated anaerobically for colony isolation.

Samples from January 25, 1972, were incubated aerobically for 24 hours (3 plates) and anaerobically for 48 hours (3 plates). Aerobic samples from June 7, 1972, and June 20, 1972, were incubated 18 hours, while the anaerobic samples were incubated for 60 hours. Samples from August 16, 1972, were aerobically incubated for 18 hours, and the

anaerobic samples were incubated for 60 hours. The latter were very tiny (approximately one-half times the diameter of a period on this page), so they were placed back onto the incubator for twelve more hours.

The samples were then counted using 15x magnification provided by a Bausch and Lombe dissecting microscope with top lighting.

For each sample run, i.e., January 25, June 7, June 20, and August 16, 1972, there were 5 sediment subsamples representing the top 5 cm and 5 subsamples representing the lower 5 cm. Thus, a vertical profile of 10 cm in length was characterized with a total of 20 core samples. Table 3 shows the number of colonies per gram of sediment when averaged over all five cores. A full compilation of the data is presented in Appendix C.

TABLE 3

SUMMARY OF BACTERIA CONCENTRATION AND CARBON CONCENTRATION IN LAKE THUNDERBIRD SEDIMENT

Sampling Date	Number of Colonies per gm	Percent Carbon
January 25, 1972		
top 5 cm	8.15×10^5	7.49
lower 5 cm	1.02×10^6	6.99
June 7, 1972		
top 5 cm	3.03×10^6	9.91
lower 5 cm	3.17×10^6	9.22
June 20, 1972		
top 5 cm	7.14×10^5	7.23
lower 5 cm	8.85×10^5	7.24
August 16, 1972		
top 5 cm	8.65×10^5	7.80
lower 5 cm	9.26×10^5	7.15

The percent carbon shown in Table 3 was also averaged over all five core samples analyzed per sampling run. This value was determined by carefully washing the sediment samples remaining in the 125-ml flasks into preweighed, evaporating dishes. The liquid was evaporated at 70°C and then oven-dried at 105°C for 24 hours to remove the water content. The dishes were cooled in a dessicator and weighed to determine the total weight of the sediment sample. Next, the dishes were ignited at 600°C for 1 hour, cooled in a dessicator, and then weighed. The loss of weight upon ignition was taken as a measure of the carbon content of the sediment. The percent carbon was readily attained by dividing the weight of the organic carbon component by the total oven-dried sediment weight.

Statistical analysis of this data was not warranted due to the limited number of samples acquired each sampling trip. The purpose was to acquire sufficient data in order to determine certain trends of the bacterial community stability in the lake sediments and of sediment carbon.

It was felt that a comprehensive survey similar to the one described was needed for the entire lake. The data should be acquired periodically throughout the year with sufficient samples collected to allow statistical comparisons of seasonal differences.

Bacteria Identification

It is important to state at this time that vigorous culturing and consequent identification of every different type of colony found on the plates was virtually impossible. An attempt was initially made

to do this, but it was then felt that this endeavor would end in frustration. Hence, the major or dominant colonies from all plates incubated were isolated, and consequent identification to genus was attempted. The colonies that were successfully identified are reported in the following discussion. These genera of bacteria were found on all plates incubated. No correlation with the depth of the sediment in which the colony was initially isolated was made. For this research, it was only deemed necessary to characterize as many of the genera of heterotrophic bacteria as possible and evaluate the qualitative stability of the dominant genera from winter through summer. Much more work is needed; hence, future investigators should consider a similar effort as shown here and possibly answer some of the basic questions which these results have raised.

Those individual colonies which exhibited distinct morphological characteristics upon initial isolation were aseptically removed with a sterile wire and streaked on sodium caseinate agar for isolation. After incubating the aerobic cultures for 18 hours and the anaerobic cultures for 48 hours, the colony morphology was redetermined. Only those colonies which had the same colony morphology as that observed initially were further characterized. The colonies were then transferred to tubes containing sodium caseinate agar and tubes containing sodium caseinate broth. These colonies were appropriately marked as to identity and age. All further analyses were carried out from these tube cultures. Once a week, the colonies were reinoculated on new media in order to keep viable young cultures always on hand until all tests were completed.

A procedure had to be developed in this work so that the key characteristics of the bacteria could be rapidly determined and thus classified by genus based on the key of V. B. D. Skerman (1967). Likewise, after these bacteria were analyzed, the characteristics were compared and those which had similar characteristics and which could be keyed to the same genus were then lumped together. These were classified as possibly being different species of the same genus.

For identification, the following determinations were made on 18-hour cultures of aerobes and 48-hour cultures of anaerobes: colony morphology, cell morphology, gram reaction, Wirtz spore stains, carbohydrate fermentation, litmus milk reaction, peptone reactions, hydrogen sulfide production, motility, indole formation, and catalase reaction. The biochemical characteristics were determined using routine bacteriological fermentation and growth test methods. The results of the biochemical tests are listed in Table 4. The characteristic colony and cell morphology are listed in Table 5.

The genera listed in the following tables were readily classified using the information given. This listing does not represent every genus of bacteria in the sediment; however, these types are typically found in all of the sediment samples.

In conclusion, the sediment studies as described above were conducted to gain some basic insight into the population density of bacteria and to give an idea of the general types of bacteria that are typical throughout the year in Lake Thunderbird sediments. The relative stability of the heterotrophic bacterial concentration from winter to summer allowed for some assurance that sampling of the sediment for

TABLE 4

RESULTS OF BIOCHEMICAL TESTS OF BACTERIA ISOLATED FROM THE SEDIMENT SAMPLES

Probable Genus	G	L	F	M	P	Sim H_2S	Sim I	Lim	Cat
Bacteroides	-	-	-	-	-	-	-	-	-
Clostridium	Ag	A	Ag	d	-	+	+	d	-
Flavobacterium	A	a	A	A	-	-	-	-	-
Brevibacterium	a	-	-	-	-	-	-	-	-
Bacillus	A	A	-	-	-	-	-	pep	+
Pseudomonous	d	-	-	-	-	-	-	d	-
Mycoplana ?	-	A	-	a	-	-	-	-	-

Sugars	Biochemical Tests
G = glucose	P = peptone
L = lactose	H_2S = sulfate reduction
F = fructose	I = indole production
M = maltose	Lim = litmus milk
	Cat = catalase production

Reaction in Sugars

A = acid production

a = weak acid production

\- = no reaction

d = doubtful acid production

pep = peptonization

\+ = positive reaction

g = gas production

TABLE 5

CHARACTERISTICS OF BACTERIA ISOLATED FROM THE SEDIMENT SAMPLES

Probable Genus	Gram Reaction	Spore Stain	Colony Morphology	Cell Morphology
Bacteroides	-	-	Anaerobic, motile, very tiny, raised, transparent, smooth	Slender rods (0.5μ × 1.5μ), singles, pairs
Clostridium	+	+	Anaerobic, motile, tiny, convex, entire, transparent, casein hydrolysis	Rods (1.0μ × 3.0μ), single, terminal spore
Flavobacterium	-	-	Aerobic, medium, translucent, convex, smooth, nonmotile, yellow color in media, casein hydrolysis	Single rods (1.8μ × 2.5μ), long chains
Brevibacterium	+	-	Aerobic, nonmotile, tiny, convex, smooth, translucent	Single rods (1.0μ × 5.0μ), pairs, short chains
Bacillus	+	+	Aerobic, nonmotile, large, translucent, smooth, entire, convex	Straight rods (1.0μ × 4.0μ), spore (1.0μ × 1.3μ), single, chains
Pseudomonous	-	-	Aerobic, nonmotile, large, raised, white, smooth, yellow color in media	Straight rods (0.6μ × 1.5μ), odd shapes
Mycoplana ?	-	-	Aerobic, nonmotile, punctiform, casein hydrolysis, smooth, entire	Rods curved (0.8μ × 1.0μ), single

use in the continuous-flow apparatus could be done at any time of the year. Furthermore, the heterotrophic bacteria and fungi which were found to be present were indicative of a viable, stable, but relatively small community of sediment microorganisms. Future work is needed to understand why the heterotrophic bacteria were not present in greater numbers.

^{32}P Labelling of Algal Particulate Organic Phosphorus

The algal particulate organic phosphorus is comprised of predominantly the insoluble organic phosphorus compounds, which are the structural components of cells. The lipids (phospholipids) are examples of these types of compounds. This fraction conprises about 10 percent of the total phosphorus in the algal cells. These forms of phosphorus compounds in algal cells are more resistant to hydrolysis and degradation than are the sugar phosphates, phosphoamides, etc.

It is felt that this heterogeneous group of particulate organic phosphorus compounds in plant and animal cells are more likely to be the dominant phosphorus compounds which reach the sediment in a lake or impoundment such as Lake Thunderbird as part of the algal body. Hence, attempts were made to label these algal components with ^{32}P and thus to study the biodegradation of this phosphorus form using the continuous-flow sediment-water apparatus described later.

In order to label these cell components with ^{32}P, it was necessary to try to maintain adequate phosphorus "limiting" conditions in the algal media. The goal was to develop and maintain a substantial agal concentration but still keep phosphorus somewhat limiting in the

culture so that only a minimum amount would be stored as orthophosphate. Ideally, the phosphorus would be continually assimilated as fast as it was mineralized. With continuous monitoring of the algal growth with time, it would be possible to determine when maximum biosynthesis occurs. At this time, the algal growth should stabilize and be dependent mainly on the phosphorus concentration in the media. The other nutrients in the media must be kept in excess, and all other conditions, including lighting, temperature, and mixing, must be kept fairly constant.

The basic KNOP's media was used for the culturing of all mixed algae cultures used in this work (Appendix B). The phosphorus concentration was varied to determine what phosphorus concentration should be used to label the algae with ^{32}P. Therefore, 2 sets of 4 flask cultures were analyzed over 20 days. The data were combined for presentation. To each of eight 1000-ml Erlenmeyer flasks, 500 ml of KNOP's media was added. To the first set of 4 flasks the following amounts of phosphorus as KH_2PO_4 were added: 50 µg P/ℓ, 100 µg P/ℓ, 200 µg P/ℓ, and 400 µg P/ℓ. To the second set of 4 flasks the following quantities were added: 1000 µg P/ℓ, 1500 µg P/ℓ, 2000 µg P/ℓ, and 3000 µg P/ℓ. Next, 10 ml or approximately 20 mg of algae were removed from the stock algal culture. The stock algal culture was maintained in 20 liters of basically the KNOP's media with 1.0 mg P/ℓ as KH_2PO_4. This tank was stable, and only distilled water was added. The algae (0.5 ml volume) were added to each flask. The flask cultures were continually mixed at the same rates using a magnetic stirrer and were maintained under constant lighting as described previously.

For the next 6 days, the flasks in set 1 were monitored. Set 2 was monitored for a total of 9 days. The monitoring of the cultures included determination of algal weight, total dissolved phosphates, hydrolyzable dissolved phosphate, and orthophosphate. Three 5-ml aliquots were removed from the flask cultures, and each was filtered through an acid-washed, preweighed, 0.45-μ Millipore filter. The algal weight was then routinely determined using a Mettler analytical balance. The three 5-ml filtrates from each flask were diluted to 50 ml with phosphorus-free distilled water and were analyzed for phosphorus according to the Environmental Protection Agency's Manual for Water and Wastewater Analysis (1971). All phosphorus analyses used in this study followed the procedure outlined in this manual.

Within 3 days, the algae cultured with 50 μg P/ℓ and 100 μg P/ℓ turned brown and were discarded. After 4 days, the culture with 200 μg P/ℓ turned brown, and, after the fifth day, the culture with 400 μg P/ℓ turned yellow. These were then discarded. In set 2, the cultures with 1000 μg P/ℓ and 1500 μg P/ℓ turned brown after the sixth day. These were also discarded. The cultures containing 2000 μg P/ℓ and 3000 μg P/ℓ developed a dark green algal growth. Table 6 summarizes the results of the weight determination and phosphorus analysis.

After 9 days, two 50-ml aliquots containing approximately 50 mg of algae were taken from the two cultures that supported algal growth. These samples were centrifuged at 1250 rpm for 10 minutes. The algal plugs in each centrifuge tube were extracted in a boiling water bath for one hour according to Fitzgerald and Nelson (1966). Fitzgerald and Nelson (1966) stated that approximately 60 percent of the soluble

TABLE 6

ALGAL CULTURE WEIGHT DETERMINATION AND PHOSPHORUS ANALYSIS

	2000 µg P/ℓ			3000 µg P/ℓ		
Day	Algal* Weight mg	Total P (µg/ℓ) Dissolved	Ortho P µg/ℓ	Algal* Weight mg	Total P (µg/ℓ) Dissolved	Ortho P µg/ℓ
1	0.3	876	250	0.3	1876	750
2	0.9	660	230	0.7	1254	540
3	1.3	600	200	1.0	750	360
4	2.4	500	100	1.8	400	150
5	2.5	420	120	2.9	430	160
6	2.9	380	100	3.6	350	140
7	3.3	270	40	3.7	414	130
8	3.4	160	too low	3.8	350	110
9	3.6	60	too low	4.0	200	too low

* Average weight of 3 samples.

organic phosphorus was removed from algae using this extraction procedure. The algal residue remaining after extraction and subsequent centrifugation at 1250 rpm for 10 minutes contained predominantly particulate organic phosphorus. The total phosphorus concentration in the supernate was a measure of the soluble organic phosphorus and the stored orthophosphorus.

The results of the extraction on the 2 samples of algae showed that:

2000 µg P/ℓ: The 360-mg algae culture contained 270 µg P/100 mg of which approximately 50 µg P/100 mg was extractable. Since no orthophosphorus was found in the supernate, the extracted phosphorus was a measure of the soluble organic phosphorus fraction. Assuming the 60 percent removal efficiency of the soluble organic phosphorus fraction, then another 33 µg P/100 mg soluble organic phosphorus remained in the residue. Thus, 187 µg P/100 mg in the residue was particulate

organic phosphorus. This was approximately 68 percent of the total phosphorus in the algae.

3000 µg P/ℓ: The 400-mg algae culture contained 350 µg P/100 mg of which approximately 77 µg P/100 mg was extractable. Again, no orthophosphorus was detected in the supernate. Assuming the 60 percent removal efficiency as stated earlier for soluble organic phosphorus, then another 51 µg P/100 mg of soluble organic phosphorus remained in the algal residue. Thus, approximately 220 µg P/100 mg of algae was particulate organic phosphorus. This represented approximately 62 percent of the total phosphorus in the algae.

The above assumptions and conclusions are based heavily on the work of Fitzgerald and Nelson (1966). It was not the purpose of this work to evaluate or prove the validity of the work of another reseacher.

The same procedure was repeated using ^{32}P as $KH_2{}^{32}PO_4$ as a tracer. This was done to check the extraction procedure. Using similar assumptions as before, the data obtained can be summarized. The supernate before extraction contained very little ^{32}P compared with that of the algae. Before extraction, 1 mg of algae had 17,711 cpm. The total weight of algae extracted was 40 mg, the total count rate of the algae was 708,440 cpm (counts per minute) before extraction. After extraction, 1 ml of the 40 ml of supernate had 1421 cpm. Thus a total count of 56,840 cpm was in the supernate. Assuming 60 percent removal efficiency of the soluble organic phosphorus from the residue, the total count in the residue representing particulate organic phosphorus after extraction was 620,033 cpm, based on 16,870 cpm per milligram after extraction. This represented about 79 percent of the total activity

in the algae as particulate organic phosphorus.

In summary, the Fitzgerald and Nelson (1966) extraction procedure seemed to be reproducible.

There was no identification of compounds implied in this extraction procedure. This was strictly an operationally defined fractionation. Also, the algae were not cultured under sterile conditions. Thus, bacteria, fungi, etc., could all be present in the culture and hence be extracted as well.

An important criterion was to keep phosphorus somewhat limiting in the cultures. It was found that by using the same culture techniques the algae could be grown and ready for extraction within 2 or 3 weeks.

Design of the Continuous-Flow Sediment-Water Apparatus

When the term "laboratory model" is considered, it is immediately assumed that an attempt will be made to simplify an entire ecosystem. An ecosystem is generally recognized as being comprised of four basic constituents: (1) abiotic substances, basic elements and compounds of the environment; (2) producers, largely autotrophic organisms; (3) consumers, heterotrophic animals utilizing particulate organic matter; (4) microconsumers, heterotrophic animals, mostly those bacteria and fungi that break down complex compounds of dead organisms, absorb some of the decomposition products, and release simple substances usable by the producers. A laboratory model which attempts to include all 4 ecosystem components can possibly give some insight into the overall general mechanisms of an ecosystem. It seems

that a closer look ought to be made at examining the ecosystem from inside, i.e., a look should be taken at how particular biological communities and physical phases interact within the ecosystem.

A good example of this are the phosphorus cycling studies in lakes, aquaria ecosystems, and algal cultures, that have been mentioned in this paper. Most nutrient cycling studies have tried to show gross comparisons between an aquarium ecosystem and a lake or impoundment ecosystem. These studies are very necessary, but it would seem that some work is necessary in developing ways in which particular compartments of an ecosystem could be modelled. It must be remembered that all modelling of ecosystems in laboratories must be validated in the field if it is to be truly adequate.

The continuous-flow cultivation techniques, as typically used in microbiological work as well as batch culturing techniques have yielded some very valuable information relating to organism growth and particular nutrient turnover studies.

The work to be described here uses a combination of the above techniques to study the mineralization and/or release of ^{32}P from the sediment to the overlying water. This is not designed to be a kinetic study of ^{32}P. However, it is an attempt at developing a technique whereby a dynamic system can be used to maintain the sediment-water and the associated sediment-biological community for substantial periods of time. This would allow for degradation studies of a particular form of organic ^{32}P compounds to be monitored as the ^{32}P is mineralized and released out of the sediment and into the overlying water.

Admittedly, in a lake's sediment-water system, the chemical, physical, and biological components interact; however, an overall steady state condition many times prevails. This steady state is resistant to change. Trying to simplify this system is at best very difficult. However, attempts are needed in order to study the sediment-water system in a laboratory situation.

Figure 3 represents an apparatus for studying algal particulate organic ^{32}P degradation at a sediment-water interface by chemoheterotrophic organisms inhabiting the sediment. This is a schematic diagram of the general apparatus. There is a total of 8 columns and 4 media reservoirs. The basic apparatus consists of the following parts: a feeding mechanism and a Sage 4-channel peristaltic pump to meter the rate of water flow to the cultivation chamber, a polycarbonate tube containing the sediment core, a constant-temperature water jacket around the polycarbonate tube, and an effluent receptor. The peripheral equipment includes a nitrogen gas source, a constant-temperature water bath, and an ice jacket for surrounding the effluent receptor. A thermometer is inserted through the rubber stopper and into the water jacket for monitoring the water temperature.

The reservoirs containing the sterile feed water are 4000-ml Erlenmeyer flasks. The rubber stoppers contain a minimum of 2 holes through which 0.25-inch-inside-diameter glass tubes are extended. Smaller diameter glass tubes are passed through the larger tubes and into the feed water. An 8-inch gum rubber tubing is used as a bacteria barrier to attach to the 0.25-inch-inside-diameter glass tube. To the glass elbow on the flask is attached a 0.45-μ filter in a

Millipore filter holder. A variable speed Sage 4-channel peristaltic pump is used to pump the feed water to each column at a constant rate of flow through each channel. Two 4-channel pumps are used along with a maximum of four 4000-ml Erlenmeyer flasks.

The cultivation chambers consist of a polycarbonate tube (core sampler, Appendix A) sealed into a larger 2-inch-outside-diameter glass tube. The larger glass tube serves as the water jacket (Figure 3). Each end of the water jacket is sealed with rubber stoppers and Du Pont silicone rubber sealer. Three holes are placed in the top rubber stopper of the water jacket for a thermometer to monitor the water temperature, for a 0.25-inch-inside-diameter glass tube which passes through the top rubber stopper of the cultivation chamber, and for an 8-inch-long, 0.25-inch-inside-diameter bent glass tube which serves as the effluent exit port for the constant-temperature water jacket. Two holes are placed in the bottom rubber stopper of the jacket, one for the 0.25-inch-inside-diameter glass tube serving as the effluent tube from the cultivation chamber and the other for a bent glass tube which is connected by tygon tubing to the water jacket's pump and serves as the water inlet to the water jacket.

Inside the glass tube, passing through both the top rubber stopper of the jacket and the top rubber stopper of the polycarbonate cultivation chamber is another small-bore glass tube which connects to the tubing from the peristaltic pump and which extends down to the sediment-water interface. The gum rubber tube, through which the small-bore tygon tubing passes and connects to the small glass tube, is attached to the large-bore glass tube located in the water jacket's

top rubber stopper. On the other end is attached a Y-connector (Figure 3). The connector is sealed on one end by using a No. 0 rubber stopper. This stopper has a tiny hole through its length in which the media tubing is passed. This has to be a tight fit. One end is hollowed partially so that the Y-connector can slip in with a snug fit. After passing the media tubing through the arm of the Y-connector, it is slipped through the gum rubber tubing and attached to the small glass tube leading into the cultivation chamber. All media tubing from the Y-connector is enclosed by the gum rubber tubing.

To maintain anaerobic conditions in the space above the water level in the cultivation chamber (Figure 4), compressed nitrogen gas is used. The compressed gas brought to the cultivation chamber is filtered in a line filter containing sterile cotton. It is then passed through tygon tubing which is attached to one arm of the Y-connector. The nitrogen gas passes through the gum rubber tubing and into the cultivation chamber. The gas passes out of the cultivation chamber *via* the overflow tube and into the effluent chamber. It then passes out of the effluent chamber and on to the next cultivation chamber *via* tubing connected to the next Y-connector in succession.

The water jacket around the cultivation chamber is wrapped with aluminum foil to keep light out. This is to ensure that heterotrophic activity will be dominant over autotrophic activity as well as to better simulate the natural condition.

The effluent chamber is contained in an insulated ice container. The chamber consists of a 60-ml pyrex glass tube. It is sealed at the top with a No. 8 double-bore rubber stopper. The

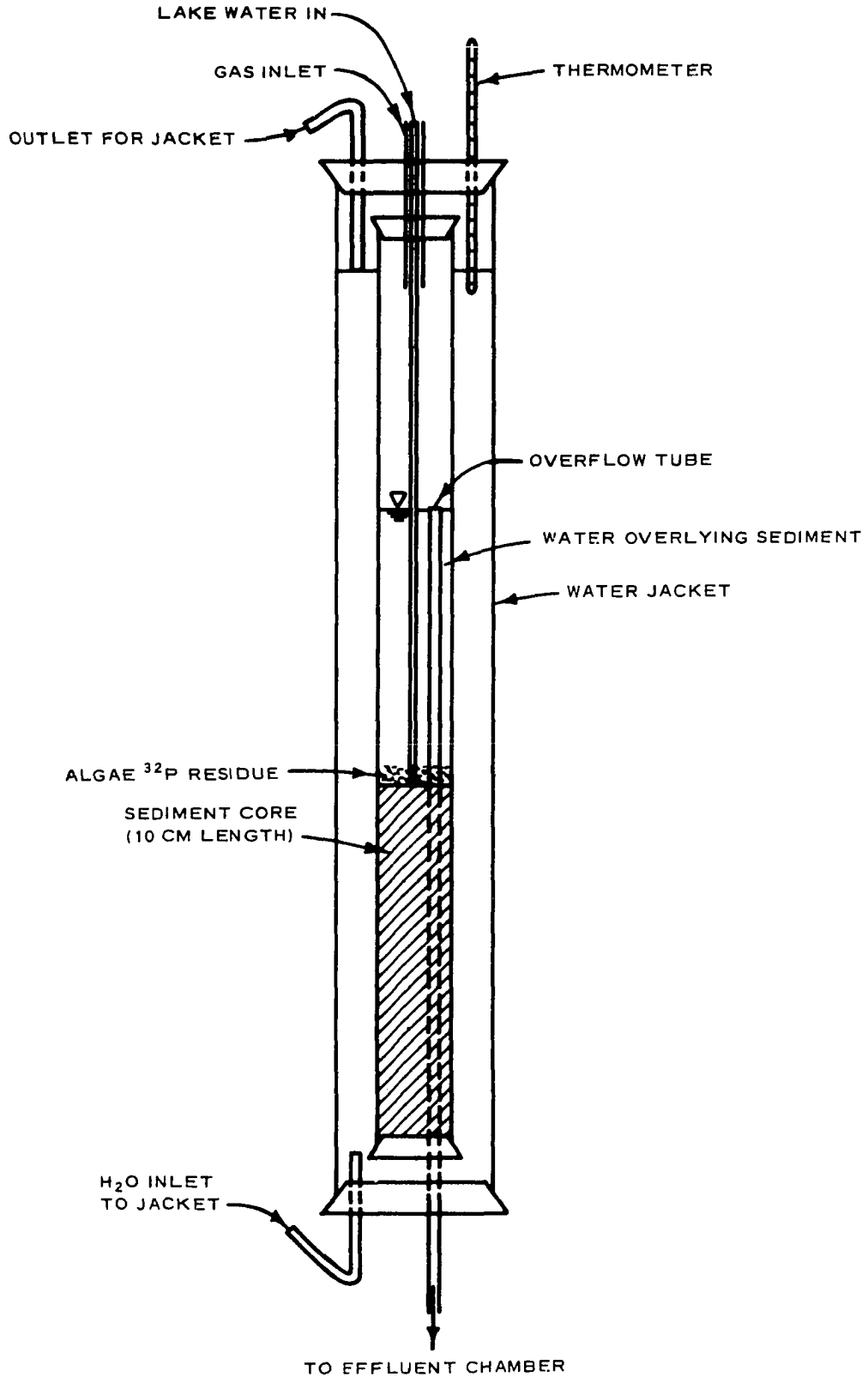


Figure 4. Cultivation Chamber

overflow tube and the gas outlet tube pass through this rubber stopper and the one on the insulated container. The inlet glass tube, which is connected to the cultivation chamber's overflow tube via a small piece of gum tubing, extends to the bottom of the effluent chamber. The gas outlet (glass) tube is either connected via rubber tubing to the next column under anaerobic operation or has a 6-inch piece of gum rubber tubing attached to it under aerobic operation. This small piece of tubing is bent to prevent airborne microorganisms from entering the chamber.

The water jackets of all eight columns are supplied by a recirculating constant-temperature water bath. The water is pumped from the bath to the water jackets and back to the bath. The temperature in the jackets is held constant at 18 $\pm$ 1°C.

Operation of Columns

The general procedures in setting up the columns in the continuous-flow sediment and water system were followed for both experimental runs. Prior to acquiring the sediment cores, an algal culture was developed following the procedure as stated earlier for the 2000 µg P/ℓ culture. Approximately 2 to 3 days prior to going to the field to get sediment samples, the culture was spiked with ^{32}P as $KH_2{}^{32}PO_4$.

The same day in which the sediment cores were to be placed in the apparatus, 8 aliquots of the culture representing approximately 17 to 20 mg of algae were extracted according to Fitzgerald and Nelson (1966). The residue representing the particulate organic ^{32}P cell

components was refrigerated until time to place them in the columns at the sediment-water interface. Four of the 8 samples were sterilized in an autoclave for 15 minutes. These were to be used in the first run only.

In the first run, the 8 sediment core samples were extruded from the polycarbonate tube until only the top 10 cm of sediment remained in each tube. While 4 of the cores were refrigerated, the other 4 cores were prepared for sterilizing in an autoclave. Each of these 4 cores was plugged on the lower end with a rubber stopper, while the other end was covered with aluminum foil. The surface of the sediment in each tube had a small volume of water covering it. This was to prevent the possibility of drying the surface while sterilizing at 121°C and 18 psi for 20 minutes.

The lake water and distilled water which were used in this run, as well as all rubber stoppers, etc., were sterilized in the autoclave. The 3 ℓ of water in the 4-ℓ Erlenmeyer flasks had been filtered through 0.45-μ filters. The rubber stoppers and glass tubes which were to be used with these flasks were put on the flask sealed with silicone rubber prior to autoclaving for 1 hour. The tubing leading from these flasks to the columns was cleaned by pumping disinfectant through it for 15 minutes, followed by 70 percent ethyl alcohol, and finally sterile, distilled water.

In other words, all tubing and glassware coming into direct or indirect contact with the sediment during the operation of the columns were sterilized prior to setting up the apparatus.

The 4 sterilized sediment cores were carefully removed from the

autoclave, cooled, and set up in the apparatus as indicated in Figures 3 and 4. The labelled algal particulate organic ^{32}P samples were aseptically deposited on the sediment in each tube.

The 4 natural sediment cores were also set up in the apparatus as in Figures 3 and 4, and the algal particulate organic ^{32}P was deposited on the sediment.

After setting up the 8 columns, 50 ml of sterile lake water were pumped into columns 1-4, and 50 ml of sterile, distilled water were pumped into columns 5-8. A waiting period of 12 hours was allowed for sediment particulates, etc., in the water overlying the sediment to settle out prior to operating the columns as a continuous-flow system.

In this first run, the system was aerobic above the water level in each column. All columns were operated at a flow rate of 2 ml/hr, and a hydraulic retention time of 24 $\pm$ 0.5 hours for each column was used. The water jacket temperature for each column was held constant at 18 $\pm$ 1°C. Columns 1 and 2 were natural; columns 3 and 4 were sterile; columns 5 and 6 were natural; and columns 7 and 8 were sterile. Column 8 began leaking water from the jacket into the cultivation chamber; therefore, the data from this column were disregarded as not being representative of a sterile system. These data are not reported in this paper.

The media reservoirs had to be refilled on days 15, 29, and 37. On day 51, lake water was prepared for columns 1-4 and columns 5-8 were disconnected. Each time water was prepared, the same chemical analyses were made on the media (Appendix D) initially as those made on the columns' effluent every 24 hours.

From day 1 through day 4, a slightly visible clay turbidity was noted in all of the column effluents. This can be attributed to the clay which remained on the sides of the polycarbonate tubes as the core was being extruded to 10 cm in length. Between day 10 and day 15, the algal particulate organic ^{32}P residue in columns 1 and 2 turned black. The residue in columns 3-7 remained green for the entire duration of the run.

On day 30, a few milliliters of formalin were added to sterile columns 3 and 4. This was done to see what effect formalin might have on the sediment and algal particulate organic ^{32}P residue. At this time, formalin was being considered as a possible "sterilizing" agent for the sediment.

Starting on day 37, nitrogen gas was purged through column 1, and on day 38, it was purged through column 6. Since the redox potential was not indicating anaerobic conditions in the water as expected, there was suspicion that the water was being oxygenated as it passed out the overflow tube. By making the space anaerobic above the water level in the cultivation chamber, a more accurate redox potential of the water could be measured.

At the conclusion of day 60 and during dismantling of the columns, a distinct reddish-brown precipitate was noted around the inside walls of the overflow tube on column 2. Column 1 had a slight coloration in the same location. This did not exist in any of the other columns.

For the second experimental run, the same general procedures were followed as those for the prior run. The main difference was

that the specific activity of ^{32}P in the algal culture was substantially increased. Columns 1 and 2 were continued from the last run, and 6 more columns were set up in the apparatus.

Columns 1 and 2 were reinoculated with more of the algal particulate organic ^{32}P residue. The remaining 6 columns were inoculated with similar amounts, i.e., 17 to 20 mg. Each of the 8 cores were set up to run under anaerobic conditions by purging with filtered, compressed nitrogen gas. Furthermore, all columns were natural with sterile lake water passing through each column.

Columns 2, 4, 6, and 8 were operated with lake water which contained an additional 0.5 mg P/ℓ as KH_2PO_4.

The media reservoirs containing lake water had to be refilled on day 16 for columns 5-8. On day 31, the lake water had to be refilled for all of the columns. The initial chemical analyses (Appendix D) of this water were the same as those made on the columns' effluent every 24 hours.

The HRT's were varied for this run. Columns 1-4 had an HRT of about 24 $\pm$ 0.5 hours at a flow rate of 2.0 ml/hr; thus, a total water volume of approximately 50 ml passed over the sediment every 24 hours. Columns 5 and 6 had an HRT of 12 $\pm$ 0.5 hours with a flow rate of 4.0 ml/hr; thus, a total water volume of 50 ml passed over the sediment every 12 hours. Columns 7 and 8 had an HRT of 6 $\pm$ 0.5 hours at a flow rate of 4.0 ml/hr; thus, a total water volume of 25 ml passed over the sediment every 6 hours.

The operating temperature for this system was kept constant at 18 $\pm$ 1°C for the entire 45 days.

The effluent chambers were sterilized as they were for the prior run. A few milliliters of mineral oil were added to each prior to sterilization in the autoclave. The mineral oil was used to reduce reaeration to a minimum while the sample was being collected.

From day 1 through day 4, a very slight clay turbidity was noted in columns 3-8. This was similar to that observed in the prior experimental run. Between days 7 and 10, the reinoculated algal residue in columns 1 and 2 had turned black. Between days 10 and 14, the residue in columns 3-8 turned black. No other visible changes in the system occurred over the duration of this run.

Analytical Methods

The effluent from each of the cultivation chambers in the continuous-flow sediment and water system were analyzed for the following parameters:

(a) Redox potential (Eh)

(b) pH

(c) Sulfate (SO_4^{2-})

(d) Iron (Fe)

(e) Manganese (Mn)

(f) Total phosphorus

(g) ^{32}P

The redox potential (Eh) was determined every 24 hours after allowing a 15-minute equilibration time for a platinum electrode. This determination is a relative measurement and is not an absolute value. The pH was measured using a Photovolt pH meter. Sulfate on 25 ml of

effluent was determined using the procedure in Standard Methods for the Examination of Water and Wastewater, Thirteenth Edition (1971). Iron and manganese were analyzed under standard conditions using atomic absorption, either a Perkin-Elmer Model 303 or a 403 Atomic Spectrophotometer. The samples were acidified when collected with 1:1 nitric acid and distilled water prior to iron and manganese analyses. Total phosphorus and total dissolved phosphorus were determined regularly on 25 ml of effluent according to the procedure in Methods for Chemical Analysis of Water and Wastes, EPA (1971). This list of parameters was restricted because of the small effluent volumes. Therefore, those parameters which best indicated the state of the sediment-water system were measured regularly.

The radioactive phosphorus was determined at least every 24 hours on the 50-ml effluent volumes, representing 24-hour composite samples, using a liquid scintillation system. A Beckman LS-133 liquid scintillation counter was used to count the ^{32}P in a standard Cocktail D fluor solution. Two milliliters of the effluent were mixed with 10 ml of the Cocktail D solution. The results were corrected for decay to day 0 and represented counts per minute per 2 ml of unfiltered effluent.

The total bacteria concentration was determined infrequently. Using the membrane filter (MF) technique, they were incubated aerobically and anaerobically at 25°C for 24 hours and 48 hours on total broth. It was possible to check for sterility of the systems during the first run as well as check for viable bacteria washout concentrations. Washout concentrations of bacteria were not too meaningful in

this continuous-flow unmixed system. Therefore, it was decided instead to occasionally check the effluent for types of bacteria, i.e., rods and cocci. Also a small aliquot of the residue in the cultivation chamber was occasionally removed by reversing the peristaltic pump. This residue was observed microscopically. All of the viable sediment-water systems were observed to maintain fungi, rods, and cocci in great numbers. This residue was returned to the sediment-water interface by pumping back through the media tubes. These tubes were disinfected prior to reconnecting to the water reservoir. This procedure was infrequently followed during the periodic replacement of water.

Finally, ammonia nitrogen analysis was regularly performed according to the Nesslerization procedure described in Standard Methods for the Examination of Water and Wastewater, Thirteenth edition (1971). However, within less than 2 weeks after the experimental runs had started interferences were noted. Tests of this parameter were then discontinued.

CHAPTER IV

DATA AND DISCUSSION

The discussion of this research has been divided into two sections corresponding to the two experimental runs. Upon conclusion of this discussion, there will be a summary in which the possible significance of the parameters tested will be reemphasized in relation to the results of both experimental runs.

Experimental Run 1

This portion of the experimental design was concerned with the study of the biological degradation of the algal particulate organic ^{32}P components and the subsequent release of ^{32}P to the overlying water under the following conditions: First, it was necessary to distinguish between the heterotrophic mediated processes and the physical processes, e.g., washing of labelled algae out of the columns. Second, it was necessary to determine if the ambient nutrient levels in the influent feed water to the columns affected the release of ^{32}P and/or any of the other stated chemical parameters. In order to determine whether the water was being reaerated prior to being analyzed, it also was necessary to observe the response of the stated parameters when the air-water interface in the cultivation chamber was replaced with a nitrogen atmosphere. Finally, it was necessary to conduct chemical

parameter comparisons of the effluents from those columns which were treated similarly in order to evaluate the reproducibility of the apparatus.

The water jacket temperature was held constant in both experimental runs at 18 $\pm$ 1°C to represent the maximum summer hypolimnion temperature of Lake Thunderbird. The flow rate and thus the HRT were preset so as to allow for minimal sediment-water mixing during the experimental run.

Sterilized and natural sediment cores were considered for this run for obvious reasons. To distinguish the biologically mediated ^{32}P releases from the physicochemical ^{32}P release mechanisms, the sterilized columns were necessary as controls. If autolysis (enzymatic) was a major factor in releasing the ^{32}P to the water, this could then be determined. Though very improbable, it might have been possible that certain autolytic enzymes were not denatured during the algal preparation procedure. Since the ^{32}P-labelled algae had been previously extracted in a boiling water bath, the enzymatic activity associated with the algal cells should have been destroyed. Furthermore, these controls enabled visual observations of the physical changes occurring in the natural sediment columns and the physical changes occurring in the sterile columns. Finally, a chemical parameter by parameter comparison was made in the effluent of the natural and sterile columns to differentiate biologically mediated release processes from strictly physical and/or chemical release processes.

Prefiltered, sterilized lake water and distilled water were used as influent feed water to the columns in order to determine the

effects of ambient chemical composition in the water on the release of ^{32}P and on that of other chemical parameters from the sediment. It was felt that, if the sediment were active in the chemical buffering of the overlying water, there would be a greater release of phosphorus, iron, manganese, sulfate, etc., from those columns in which distilled water was used. This buffering capacity was also compared under natural and sterile conditions. The buffering action would generally be controlled by the diffusional transport of the soluble elements through the interstitial water. However, the heterotrophic community activity obviously could mediate how much and to what extent this release would occur. Also, some of these elements may be recycled within the community as fast as they are released to the interstitial water, since they are not being replenished from the overlying water.

The results of this experimental run are shown in Figures 5-18. Those columns which were operated similarly are grouped and compared in the discussion.

Columns 1 and 2

The data from columns 1 and 2 are shown in Figures 5, 6 and 7,8, respectively. As stated earlier, columns 1 and 2 contained sediment with its indigenous heterotrophic community. Sterile lake water was passed through each cultivation chamber at 2 ml/hr while maintaining a 24-hour HRT. The air-water interface in the cultivation chamber of column 1 was replaced with a nitrogen-water interface after day 37. Column 2 was maintained with an air-water interface in the cultivation chamber for the entire experimental run.

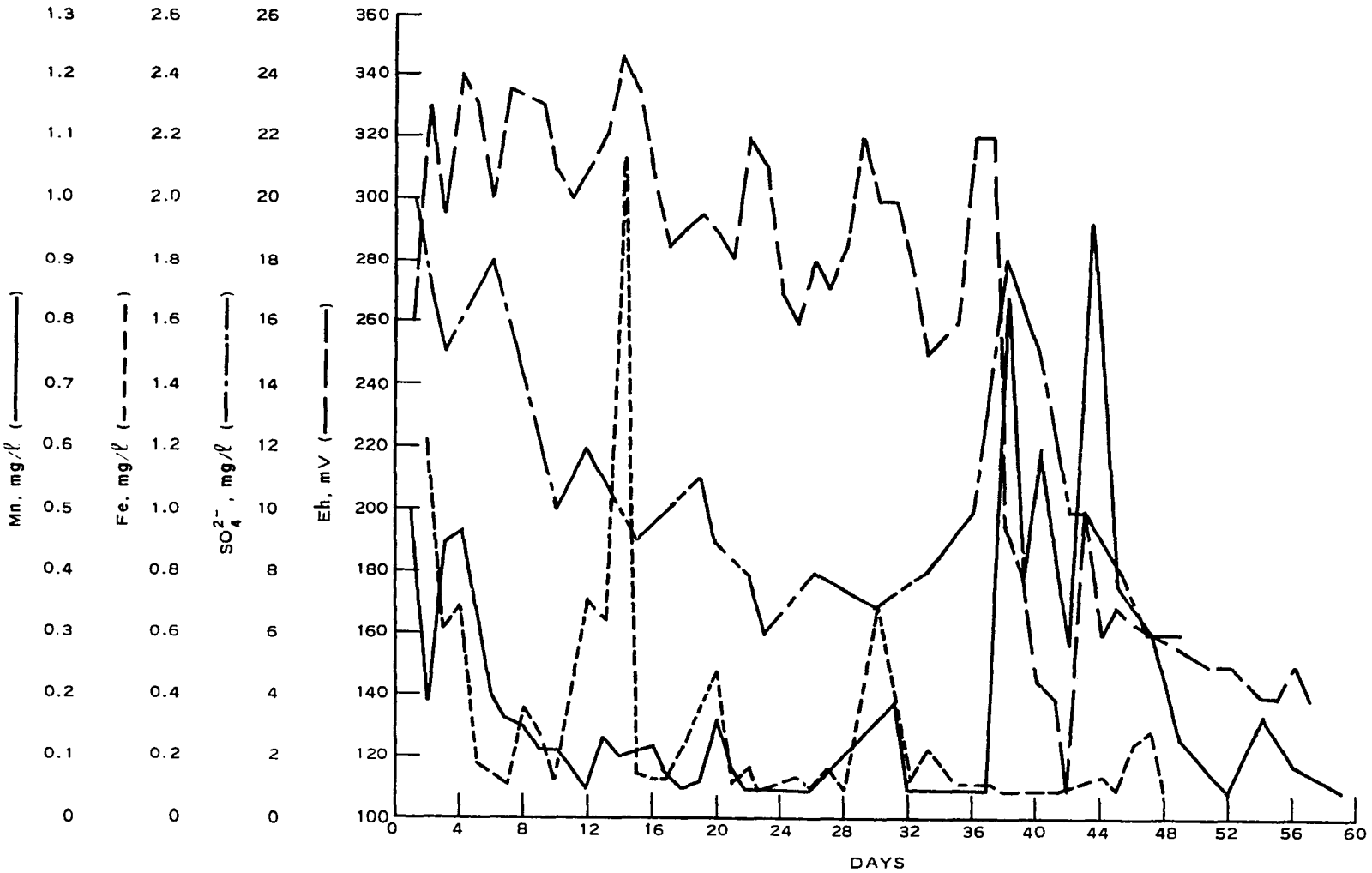


Figure 5. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 1 Effluent During Experimental Run 1

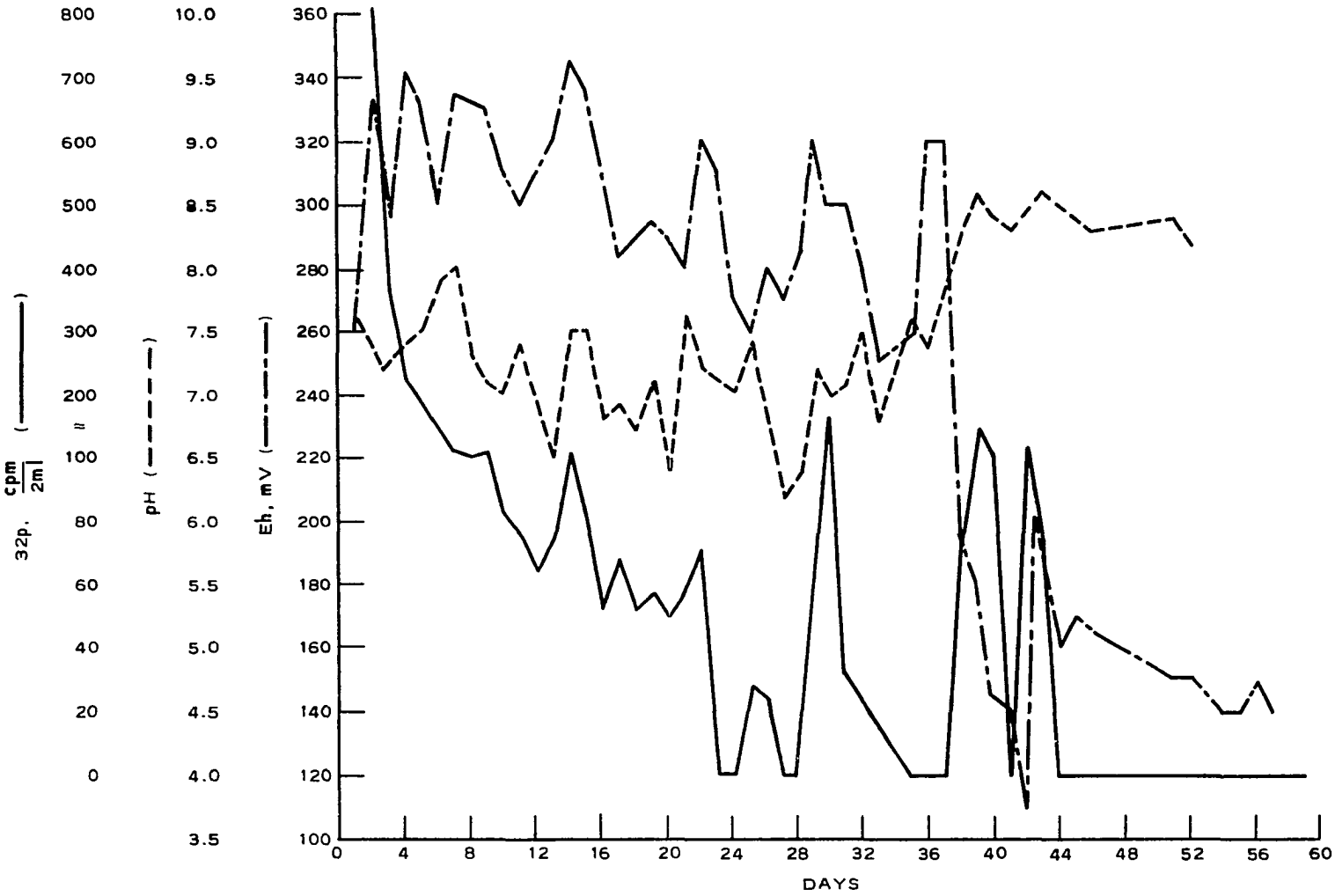


Figure 6. Comparison of ^{32}P, pH, and Eh for Column 1 Effluent During Experimental Run 1

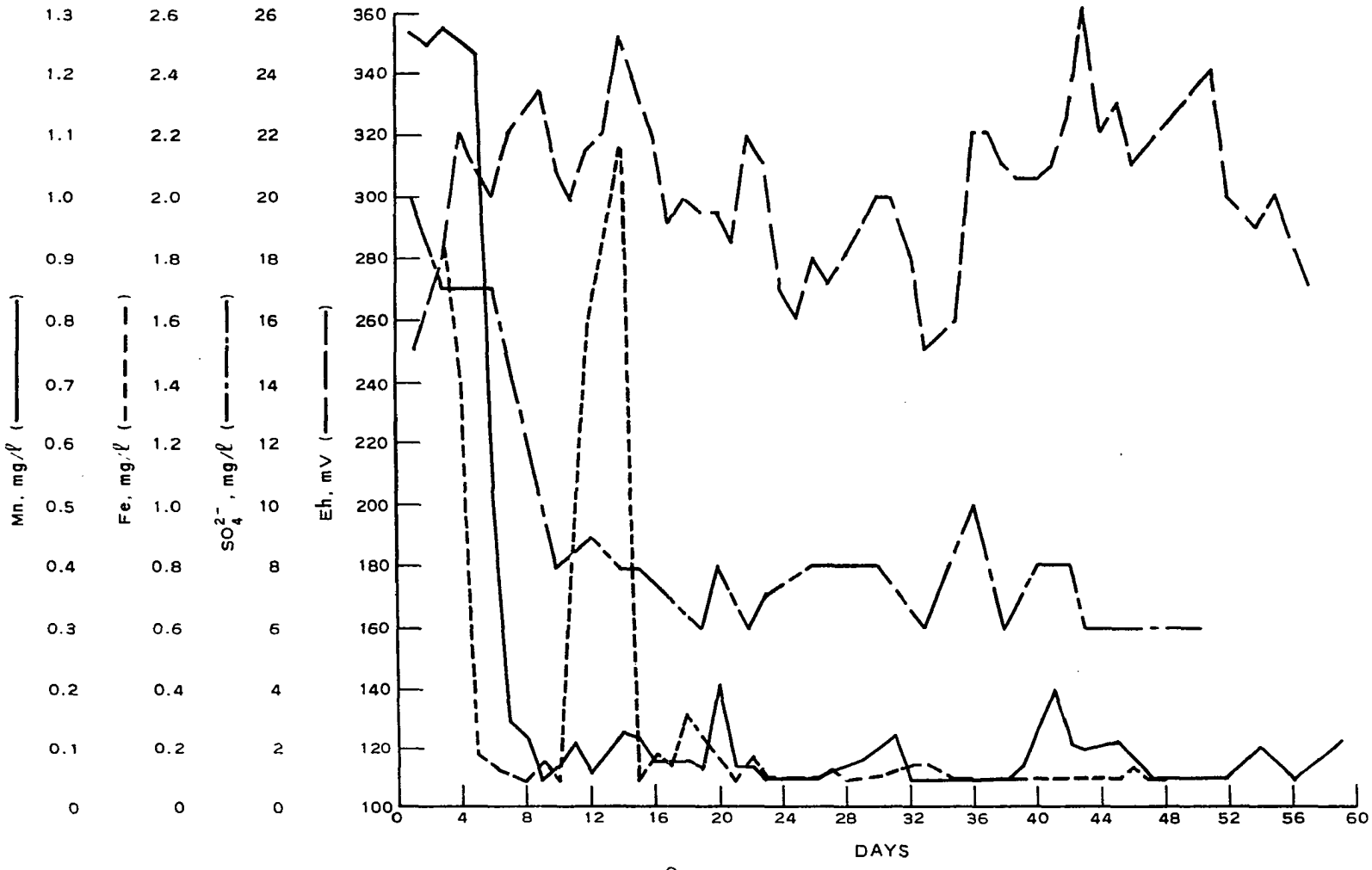


Figure 7. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 2 Effluent During Experimental Run 1

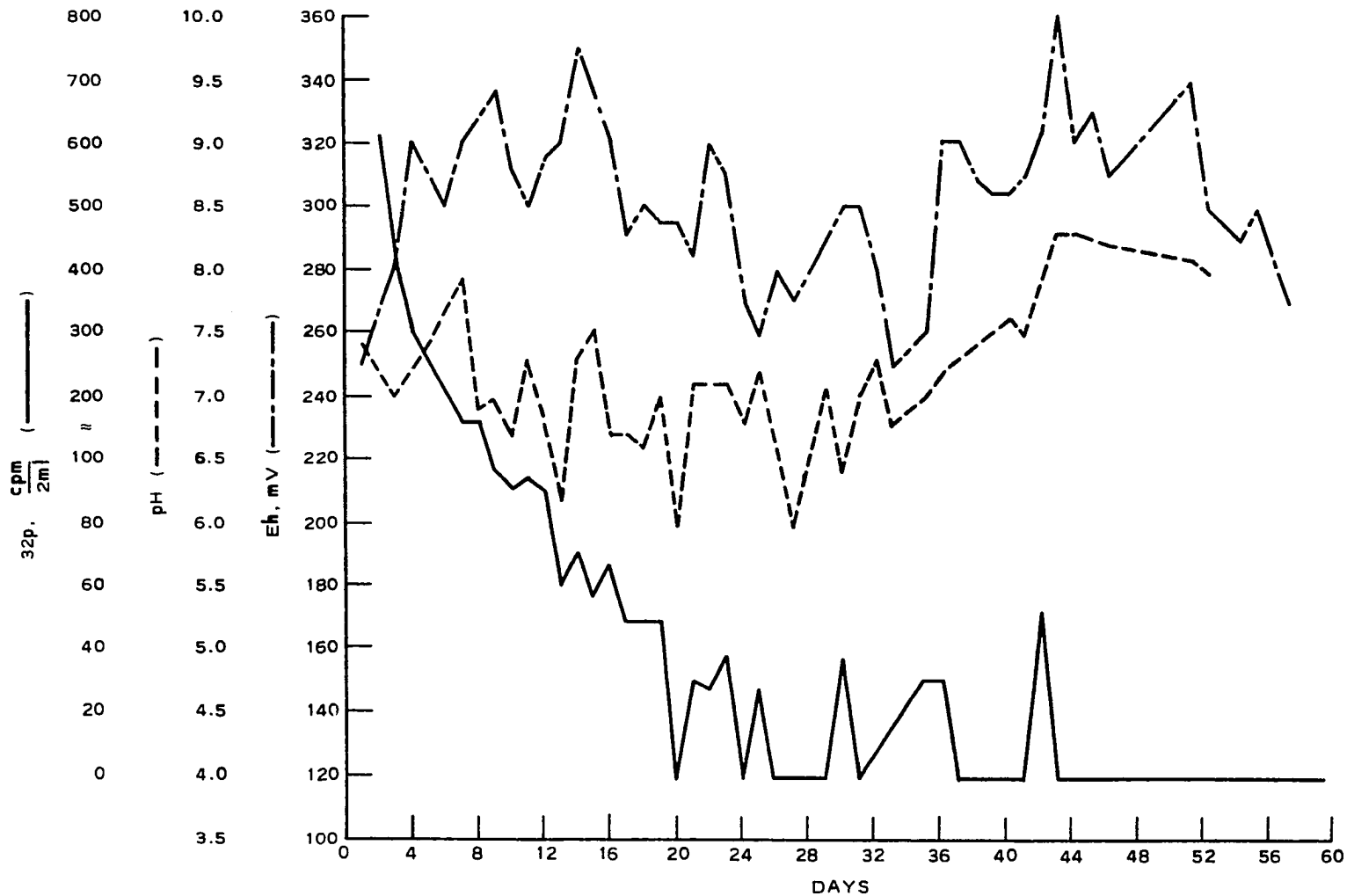


Figure 8. Comparison of ^{32}P, pH, and Eh for Column 2 Effluent During Experimental Run 1

From day 1 through approximately day 5, large concentrations of manganese (Mn) and iron (Fe) were measured in the effluent from these columns as shown in Figures 5 and 7. During this same time period, the effluent contained some clay turbidity. This turbidity was attributed to a small quantity of sediment which remained attached to the walls of the cultivation chambers during preparation of the columns. Later, the sediment was washed off the sides of the cultivation chambers as the water flowed through the system, thus causing the turbidity in the effluent and possibly the high manganese and iron concentrations that were measured. Since iron and manganese in their oxidized form as precipitates or sorbed on clay particles can pass through 0.45-μ filters, filtering of the samples would not detect their physical state. This was attempted; but about 90 percent of the iron and manganese passed the filter.

Figure 5 shows that by day 2 there was a sharp decrease in the manganese concentration to 0.2 mg/ℓ. There seemed to be a release of manganese from day 2 to day 4 to a maximum concentration of 0.47 mg/ℓ. Over the next 5 days, less manganese was released from the column. From day 9 through day 37, <0.05 to 0.20 mg/ℓ of manganese was released.

Figure 7 indicates that the manganese release pattern was very similar to that of column 1 from day 9 to day 37. The manganese concentration in the effluent from column 2 remained fairly constant from day 9 through day 59, i.e., <0.05 to 0.20 mg/ℓ. Comparing the effluent manganese concentrations of columns 1 and 2 from day 9 through day 37, a very similar pattern was found. From days 19 through 20 and days 27

through 31, only a slight increase in the manganese concentration occurred, i.e., <0.05 mg/ℓ to approximately 0.20 mg/ℓ.

Since iron and manganese react very similarly to changes in redox conditions, a similar pattern of iron release was expected for both columns through the first 37 days. Figures 5 and 7 show this to be generally true. However, the effluent manganese and iron concentrations of column 2 decreased faster then they did in column 1. This may be indicative of column 1 having more clay turbidity than did column 2 at the start of the experimental run. Since a similar pattern existed for sulfate, this may indicate that the reducing activity of the sediment biota was greater in column 2 than column 1 at the start of the experiment. Manganese is known to be reduced from Mn(IV) to Mn(II) at a higher redox potential than that for iron. Technically, sediment-manganese release from the columns should have been detected prior to the iron release. However, this would be expected only if the redox changes occurred slowly over a few days. If the redox changes were fast with respect to the HRT, the increased manganese and iron concentrations in the effluent would overlap. This apparently occurred in these columns.

From day 10 through day 15, the effluent iron concentration released from columns 1 and 2 were very similar, <0.10 to 2.1 mg/ℓ. During this same period, there was no indication of a significant manganese release. For the first 14 days, the redox conditions might have changed slowly as a result of having a relatively small initial sediment heterotrophic bacteria community of approximately 10^6 cells/gm. As the community increased in numbers, the reducing capacity

of this community would have increased as a result of increased biological activity. Thus, the sediment redox potential would have decreased slowly at first then more rapidly as the community increased in size. As this occurred, the oxidized microzone would have decreased and ultimately vanished. This would have allowed the reduced iron to move into the overlying water. If this were the case, the manganese release might have been hidden in the first few days as a result of the clay turbidity, which contains considerable manganese in the clay mineral structure. By day 11, the sediment redox potential was probably low enough so that iron could be reduced from Fe(III) to Fe(II). It is very interesting to note that the maximum iron concentration released from both columns was 2.2 mg/ℓ. This would indicate that both columns were reacting in a biologically similar manner, under the stated experimental conditions. Instrumental error was not considered since the iron standards were analyzed periodically as a check against error. Also, the iron and manganese releases in column 1 occurred during similar time periods after day 15. This was further evidence that the communities had stabilized. Figures 5 and 7 show similar manganese release patterns after day 17 and before day 38. As stated earlier, however, very little iron was detected in the effluent of column 2 after day 17 to the end of the experimental run. Whereas similar iron and manganese releases occurred in column 1 from day 17 through day 37, virtually no iron was released in column 2 from day 21 through day 48. The differences in iron release between the columns may reflect slightly different individual column characteristics, which is not surprising.

Furthermore, the iron and manganese released in column 1 after day 37 were distinctly different from the pattern prior to day 38.

On day 38, nitrogen gas was purged continuously into and out of the cultivation chamber of column 1. From the data, it was suspected that the water was being reaerated prior to passing out of the column. This could have resulted in precipitation of oxidized iron and manganese in the cultivation chambers. The large release of manganese from day 38 through day 59, shown in Figure 5, strongly suggests that manganese was indeed being precipitated out in the cultivation chamber prior to day 38. A plausible reason for the reduced quantity of iron being released from day 38 to day 48 is that most of the available sediment iron had already been released between days 10 and 15. After day 15, the formation of sulfides, which requires a lower redox potential than the reduction of ferric to ferrous iron, may have precipitated much of the available iron as ferrous sulfide. A black coloration at the sediment-water interface in both columns formed during this time period and may be an indication of ferrous sulfide formation. Furthermore, the sulfate concentration in both columns decreased substantially, thus indicating that the redox condition in the water-sediment system was much lower than the electrode potential indicated.

From day 1 through day 37, as shown by Figures 5 and 7, the sulfate $\left(SO_4^{2-}\right)$ concentration steadily decreased from 20 mg/ℓ to about 6 mg/ℓ. The decreasing sulfate concentration in both columns indicated that the redox potential at the sediment-water interface was very low. Sulfate is reduced to sulfide at redox potentials of about

-150 mV. In comparison, the sulfate concentration as measured in the effluent of column 2 decreased much faster than in the effluent of column 1. The lowest electrode potential (Eh) in the effluent measured after a 15-minute equilibration time was 260 mV. Thus, the electrode potentials as indicated in Figures 5 and 7 were not an accurate measurement of the redox conditions in the columns. These can only be interpreted in the most general way since the water was apparently being reaerated prior to the measurement of the electrode potential and chemical analysis. Figure 5 shows that when nitrogen gas was purged over the air-water interface of column 1, the electrode potential dropped to about 120 to 140 mV within 24 hours, further indicating that the effluent was being reaerated as it left the cultivation chamber. The apparent release of sulfate, noted on days 38 and 40, as shown in Figure 5, for column 1 when the electrode potential of the effluent decreased from 290 to 110 mV seemed to be anomalous. A feasible explanation is that the true sediment redox potential may have been unstable and fluctuating around the narrow redox potential of about -150 mV, which is required for sulfide formation. If the sediment redox potential was greater than -150 mV, then sulfate (-ite) would not have been reduced to sulfide and would be washed out of the column. Another explanation may involve a Mn-S complex which was precipitated at a higher redox condition on glass or plastic surfaces of the column. After purging the system with nigrogen, the redox condition may have decreased sufficiently to solubilize the Mn-S complex and thus resulted in their washout from

the column. The sulfate concentration of the effluent would have been increased in both cases.

Figures 6 and 8 graphically represent the ^{32}P, pH, and Eh trends from columns 1 and 2, respectively, as measured in their effluent. In both columns, there was a gradual decreasing release of ^{32}P from day 1 through about day 22. In Figure 6, there was a very slight increase in the amount of ^{32}P released from the algae between day 12 and day 16 from 60 to 100 cpm/2 ml. This generally coincided with the iron release depicted in Figure 5. The apparent small release between day 24 and day 27 was probably not significant because of the very low count rate involved. However, between days 28 and 34, a spike release occurred. A maximum release on day 30 of 100 cpm/2 ml coincided closely with the iron and manganese release shown in Figure 5. During this same time period, the pH of the effluent remained fairly stable between 6.5 and 8.0. Upon purging the column with nitrogen gas on day 37, the pH increased to 8.5 by day 39 and remained constant through the duration of the experiment. There was a large release of ^{32}P between day 37 and day 44. This closely corresponds to the manganese release shown in Figure 5 for this same time period.

Analogous results are also shown in Figure 8 for column 2. There did not seem to be a significant ^{32}P release from day 11 through day 15. If ^{32}P were associated with the iron release, it would have been expected to be released during this time. This possibly indicates that the phosphorus being released between day 1 and day 15 was bacterial ^{32}P or sorbed ^{32}P. The ^{32}P released during this time was particulate. This was based on the results of filtering 2 ml of

effluent through a 0.45-μ filter and counting and filtrate. All of the ^{32}P remained on the filter. Further microscopic examination indicated that none of the labelled algae was being washed out. It is not clear, however, whether the bacterial ^{32}P was indeed part of the initial extracted algal residue. From day 24 through day 59, virtually no ^{32}P was released from the column. The small spike releases of ^{32}P did not correspond to either an iron or a manganese release. Therefore, these spike releases could be a result of isotopic exchange, or the releases may be a reflection of shifting species or cycling of the indigenous heterotrophic community numbers. This would be expected. However, it was not feasible to attempt to validate this possibility because the small continuous-flow apparatus used would not permit adequate sampling at the sediment-water interface. Only on day 42 was any ^{32}P released corresponding with a possible manganese release. However, the duration of the ^{32}P release was not similar to the duration of the manganese release. This was evidence for the fact that the ^{32}P was not complexed with the manganese.

There was slightly more variability in the pH of the effluent from column 2 than in that from column 1. From day 1 through day 20, there was a slight decrease in pH from about 7.5 to a low of 6.0. The pH generally fluctuated between 6.0 and 7.2 from day 20 through day 36. Upon changing the influent water feed, the pH of the effluent increased slightly to about 8.2 on day 43, then it decreased to about 8.0 by day 52. This variability was not too unexpected considering the periodic change of the influent water with slightly different initial pH every 14 to 15 days. The pH of the influent lake water on

day 37 was 8.2 (Appendix D). The pH of column 1 and column 2 fluctuated similarly through day 52. Column 2 effluent had a slightly lower pH from day 12 through day 37. After day 37, the pH from column 1 and column 2 effluent increased slightly. The pH of columns 1 and 2 remained constant between 8.0 and 8.5 from day 39 and 42, respectively, for the duration of the experimental run.

The only physical changes which occurred in columns 1 and 2 were observed between days 10 and 15. During this time, the algal particulate organic ^{32}P residue turned from green to black. This black coloration remained for the duration of the run. It could be surmised that the redox potential at the water-sediment interface was very low. This black coloration could have been due to precipitation of vivianite or sulfides. The results of the second experimental run and the reduced sulfate concentration in columns 1 and 2 of this run would indicate that sulfides were probably present. These results will be discussed in their appropriate section.

Columns 3 and 4

The data shown in Figures 9, 10 and 11, 12 represent column 3 and column 4, respectively. Columns 3 and 4 contained sediment which had been sterilized in the autoclave at 121°C and 18 psi. During this experimental run, periodic checks on the sterility of the effluent were made by culturing an aliquot according to Millipore techniques. The aliquot was cultured under aerobic and anaerobic conditions at 25°C on nutrient broth media.

As in columns 1 and 2, the manganese and iron concentrations in the effluent of columns 3 and 4 decreased rapidly from day 1 through

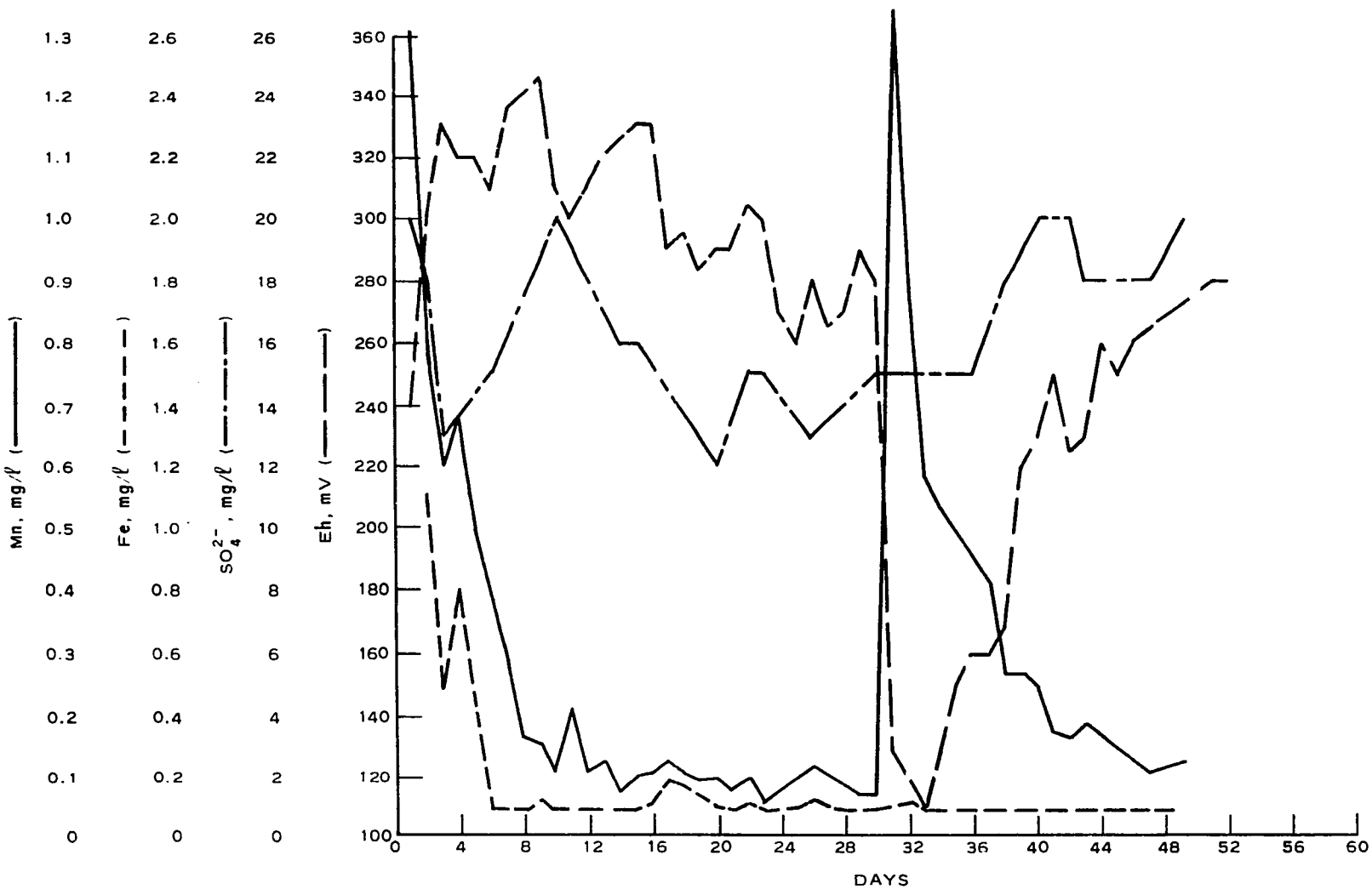


Figure 9. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 3 Effluent During Experimental Run 1

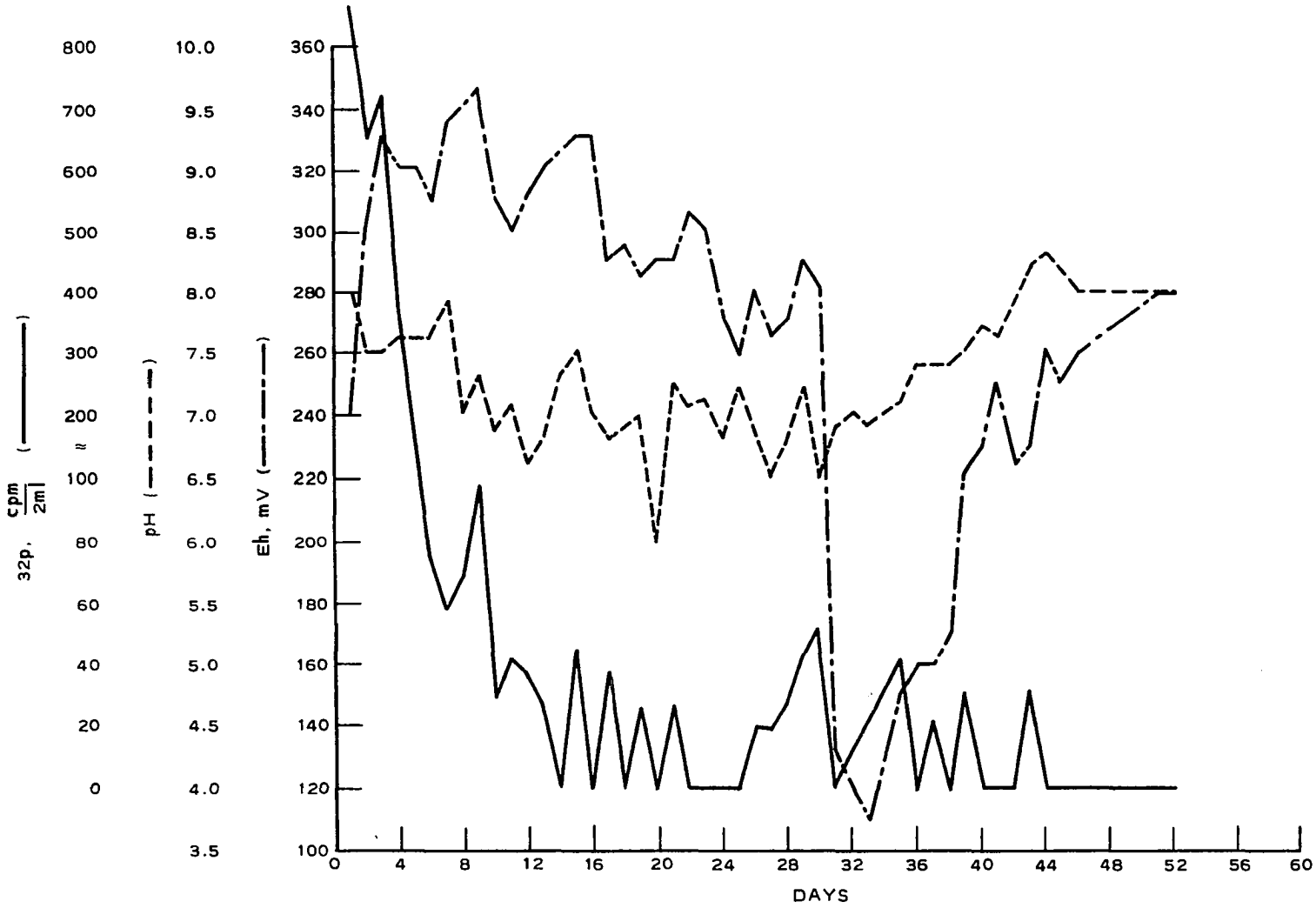


Figure 10. Comparison of ^{32}P, pH, and Eh for Column 3 Effluent During Experimental Run 1

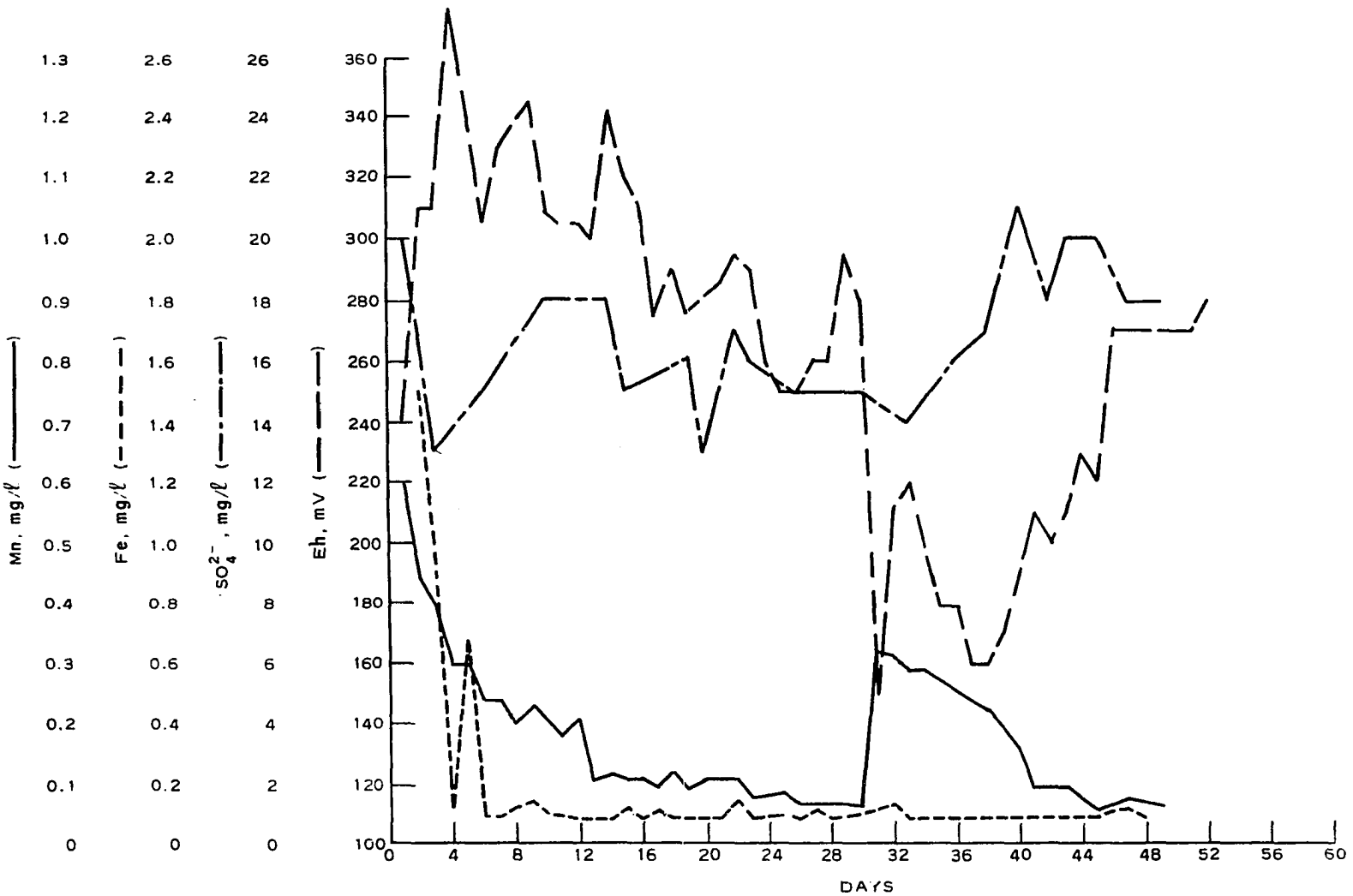


Figure 11. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 4 Effluent During Experimental Run 1

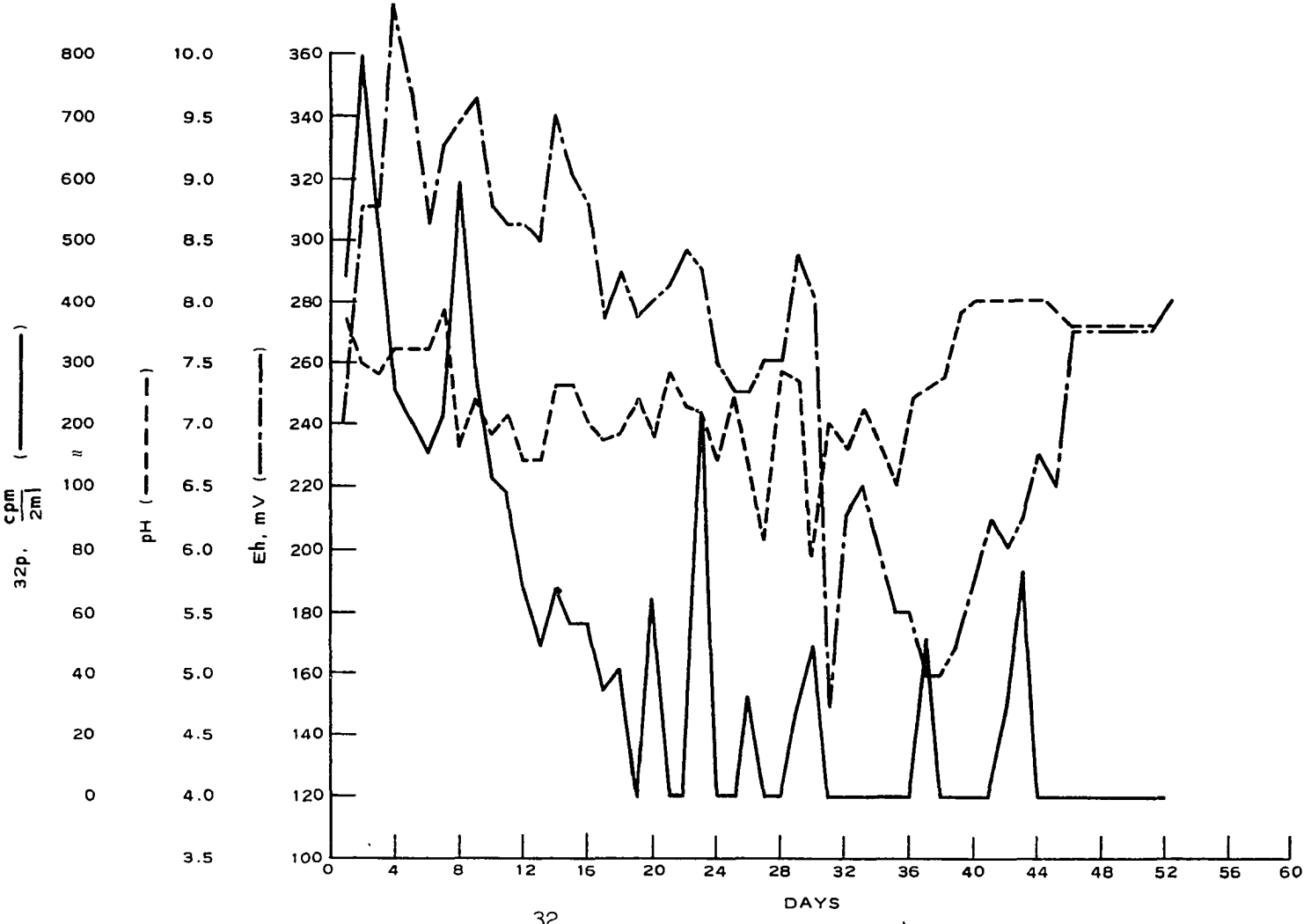


Figure 12. Comparison of ^{32}P, pH, and Eh for Column 4 Effluent During Experimental Run 1

day 6 (Figures 9 and 11). This general washout of these metals coincided very closely with the decrease of clay turbidity in the effluent. By day 13, the effluent iron and manganese concentrations from both columns levelled off at less than 0.10 mg/ℓ for iron and 0.10 mg/ℓ for manganese. These concentrations were almost representative of the influent lake water concentrations for these elements (Appendix D). The normal decrease of the redox condition in the sediment, as a result of biological activity, was not occurring. Futhermore, the sulfate concentration remained uniformly high throughout the experimental run. This would indicate that both of the column's sediments were biologically inactive for the entire experimental run.

During the entire experimental run, the sediment redox potential was never low enough to reduce the oxidized iron from Fe(III) to Fe(II). The evident manganese reduction from Mn(IV) to Mn(II) on day 31, as a result of adding concentrated formalin, offered further evidence that the redox conditions in the column were not low enough for iron to be released. Thus, the importance of the sediment heterotrophic community in mediating the release of iron from the sediment to the overlying water was vividly shown when comparing the data from columns 1 and 2 to columns 3 and 4.

Further evidence of sterile, inactive sediment in each column could also be seen in the effluent sulfate concentrations and in the electrode potentials (Eh) for columns 3 and 4. Figures 9 and 11 compare the effluent sulfate concentrations in columns 3 and 4. It was readily observed that the sulfate concentration from these columns did not decrease as it had in columns 1 and 2. However, there did

seem to be variation in the day to day sulfate levels in both columns 3 and 4. The variation in this analysis may have been a result of interferences such as organic acids. This was reasonable considering the large quantity of algae initially placed in the column and realizing that there were some soluble organic acids present with the algal residue.

Furthermore, the sterilization procedure used for the sediment may have resulted in the release of a significant quantity of soluble organic material to the water. The range in the sulfate concentration of column 3 was between 20 and 12 mg/ℓ. For column 4, the range of sulfate concentration was about the same as that for column 3 from day 1 through day 49. The fluctuations indicate a constant general trend over the entire experimental run. When compared with the sulfate concentration in the media water, the fluctuation may be explained by interferences typical of the analysis.

The redox potentials, or more accurately, the electrode potentials (Eh) for columns 3 and 4 from day 1 through day 29 are shown in Figures 9 and 11. The fluctuations shown are not too significant considering how the electrode potentials were measured. The data are shown for comparisons with other columns. A 15-minute equilibration time for the electrodes to measure small potentials is not a sufficiently long time to eliminate some drift in the measurements; hence, such fluctuations as those observed between about 340 and 270 mV may have been an expected amount of variation. However, when a few milliliters of concentrated formalin were added to both of these columns on day 30, a significant drop in the electrode potential was

observed in the effluent of both columns on day 31. In column 3, the drop was from 280 mV on day 30 to 130 mV on day 31. In column 4, the Eh dropped from about 280 mV on day 30 to 150 mV on day 31. On day 33, the Eh was down to 110 mV in the effluent of column 3; however, a significant increase in the electrode potential occurred from day 33 through day 52. By day 52, the effluent Eh measurement from column 3 had increased to 280 mV. The slow recovery was probably a result of the continued dilution of the formalin present in the column.

Upon addition of the formalin to column 3, a significant release of manganese was observed from less than 0.10 mg/ℓ to greater than 1.3 mg/ℓ on day 31. This release of manganese decreased as the electrode potential increased over the next 18 days. On day 49, the manganese concentration in the effluent was about 0.10 mg/ℓ and the electrode potential was measured to be 270 mV. During this same time, no increase in the effluent iron concentration was indicated.

In column 4, a similar pattern was observed concerning the rate of recovery of the electrode potential to what it was prior to the addition of concentrated formalin. The rapid rise in the electrode potential of the effluent, measured on days 32 and 33, was followed by another decrease, i.e., from 220 mV on day 33 to 160 mV on day 37. Three milliliters of concentrated formalin were added to this column to see if the subsequent reduction of the electrode potential would be followed by iron, manganese, and ^{32}P releases. In Figure 11, a noted release of manganese (Mn) from the column from day 31 through day 40 is evident. This expected release coincides with the decreased electrode potential. By day 46, the electrode potential had increased to

270 mV, and the Mn returned to 0.1 mg/ℓ, which are what they were prior to the formalin addition.

Figures 10 and 12 show the ^{32}P release from the algal residues, pH, and electrode (redox) potential as these parameters varied with time in columns 3 and 4. For approximately the first 22 days of operation, there was a gradual decreasing release of ^{32}P from columns 3 and 4. In Figure 10 on days 8 and 9, an apparent increase is shown in the amount of ^{32}P released from the columns. This apparent release was not associated with either the iron or manganese release shown in Figure 9. This ^{32}P release was considered to be part of the washing of soluble organic ^{32}P from the algal residue. The slight spike releases of ^{32}P on days 15, 17, 19, and 21 were not considered to be statistically significant. From days 27 to 31 and days 31 to 36, a small release of ^{32}P occurred in column 3 as shown by Figure 10. This apparent release showed up as a maximum of about 50 cpm/2 ml in the effluent. Since this release seemed to correspond fairly well with the manganese release shown in Figure 9, some of the ^{32}P may have been associated with manganese or another redox reaction. This is doubtful, however, since the absence of oxygen is generally required in order to reduce manganese oxides, MnO_2 to Mn(II). If, however, the addition of formalin on day 30 reduced the MnO_2 to Mn(II), then a complexing of soluble organic ^{32}P or any available ortho ^{32}P with Mn(II) may have been possible. Pyrophosphates are known to form insoluble compounds with Mn(II).

A similar ^{32}P release was noted from column 4 as shown by Figure 12. As stated earlier, on day 8 the effluent ^{32}P increased from

about 200 cpm/2 ml on day 7 to about 600 cpm/2 ml. This spike release was the result of the washing out of soluble organic ^{32}P associated with the algal residue. There were spike ^{32}P releases on days 20, 23, 26, 29, 30, 37, 42, and 43. These releases were probably not biologically mediated nor were they related to redox potential changes, manganese releases, pH changes, or formalin induced reactions. These spike ^{32}P releases may also have been the result of further washing of the algal residue, or the ^{32}P releases may have been a result of cellular degradation by sediment enzymes which escaped being denatured during the autoclaving process.

The pH in the effluent from both columns 3 and 4 remained constant from day 1 through day 36. Daily fluctuations between 6.5 and 7.5 were not unexpected and did not represent significant pH changes. The addition of formalin did not affect the pH of the effluent. However, upon changing the influent lake water on day 37, the pH increased accordingly (Appendix D).

Contrary to the physical changes which occurred in columns 1 and 2, there were no visible changes in columns 3 and 4. The green coloration of the algal particulate organic ^{32}P residue remained for the duration of the experimental run. This also supported the higher electrode potentials which were measured in columns 3 and 4, as well as the high sulfate concentration in the effluent from both columns.

Columns 5 and 6

The data from columns 5 and 6 are shown in Figures 13, 14 and 15, 16, respectively. Columns 5 and 6 contained sediment with the

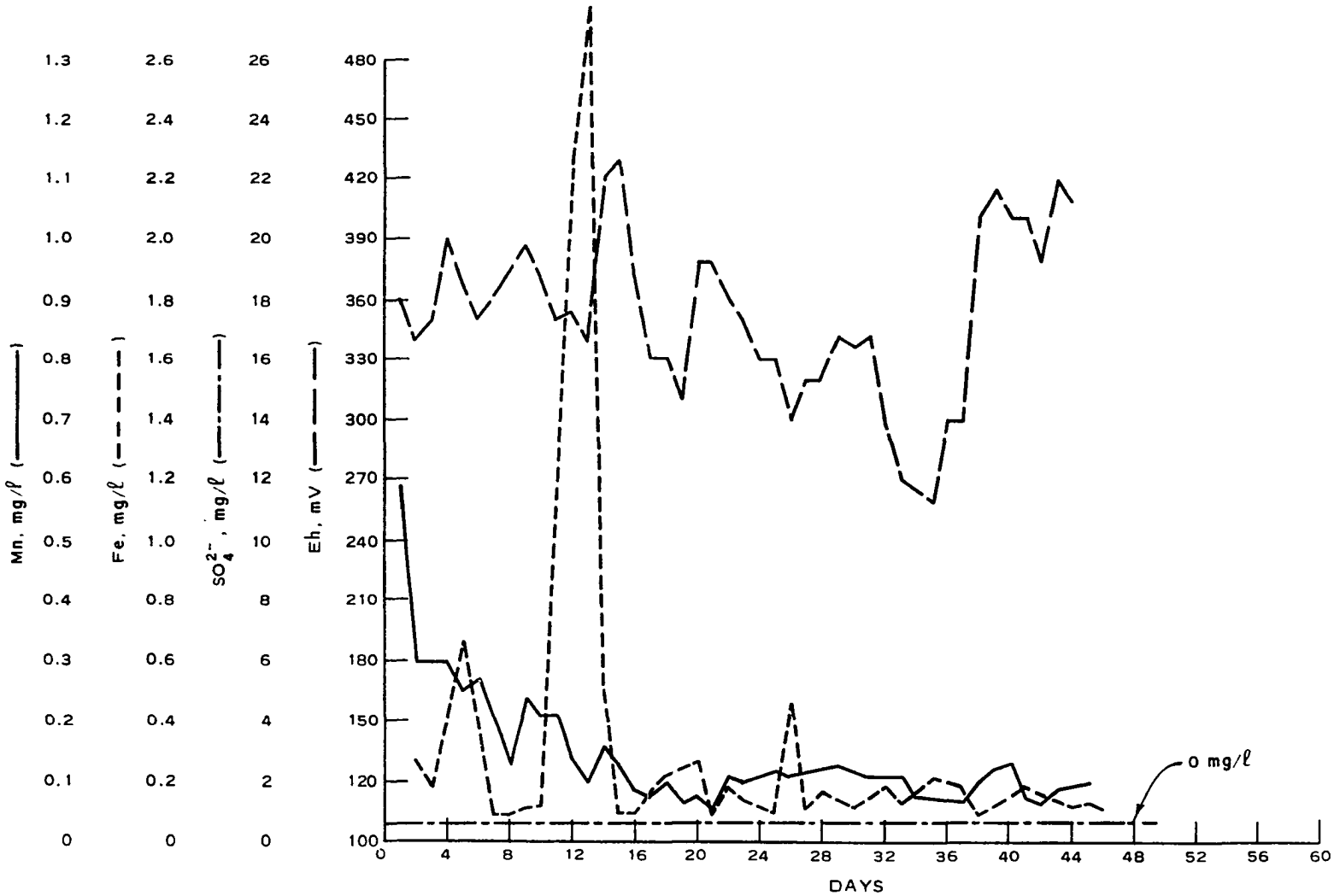


Figure 13. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 5 Effluent During Experimental Run 1

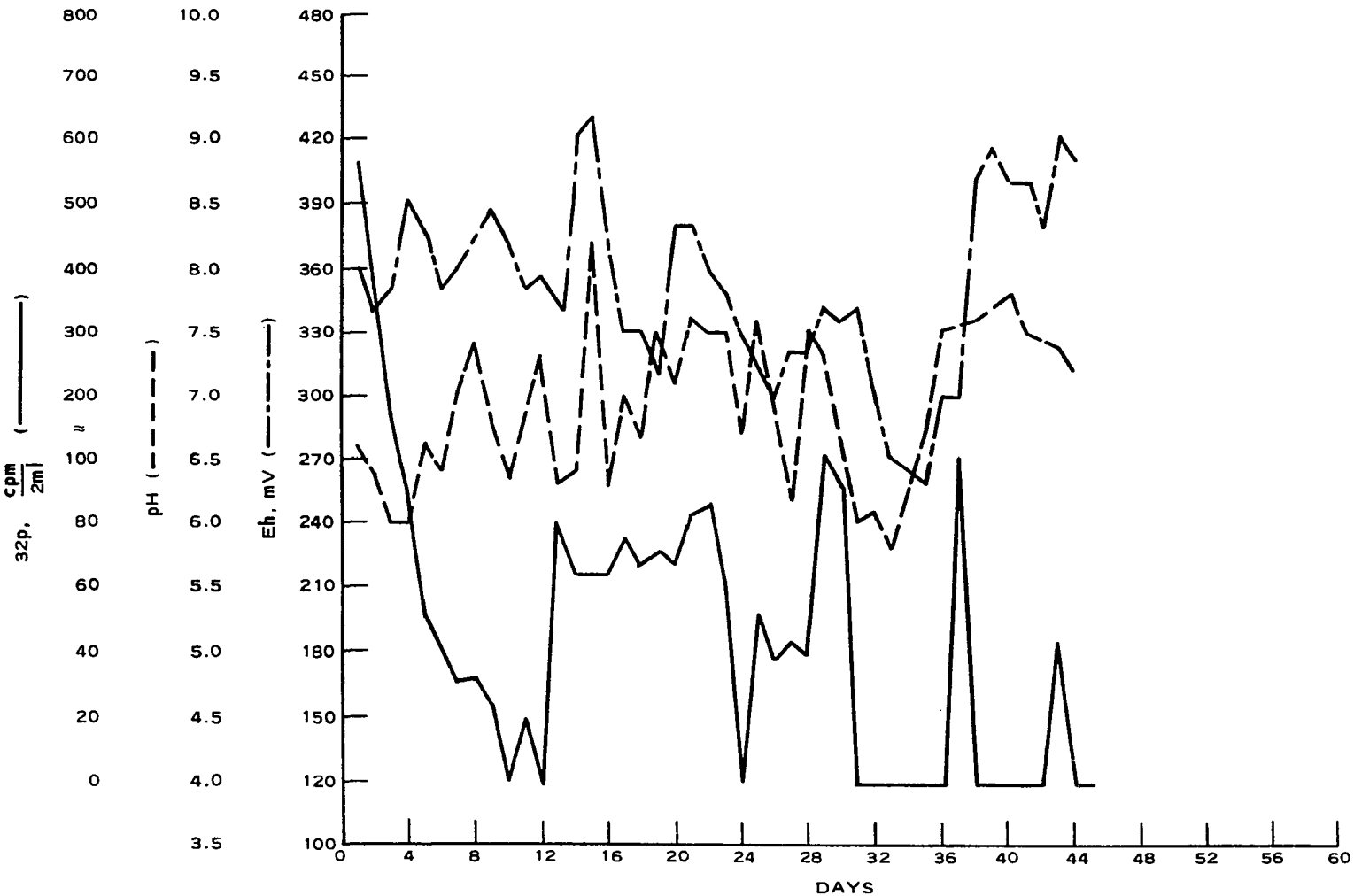


Figure 14. Comparison of ^{32}P, pH, and Eh for Column 5 Effluent During Experimental Run 1

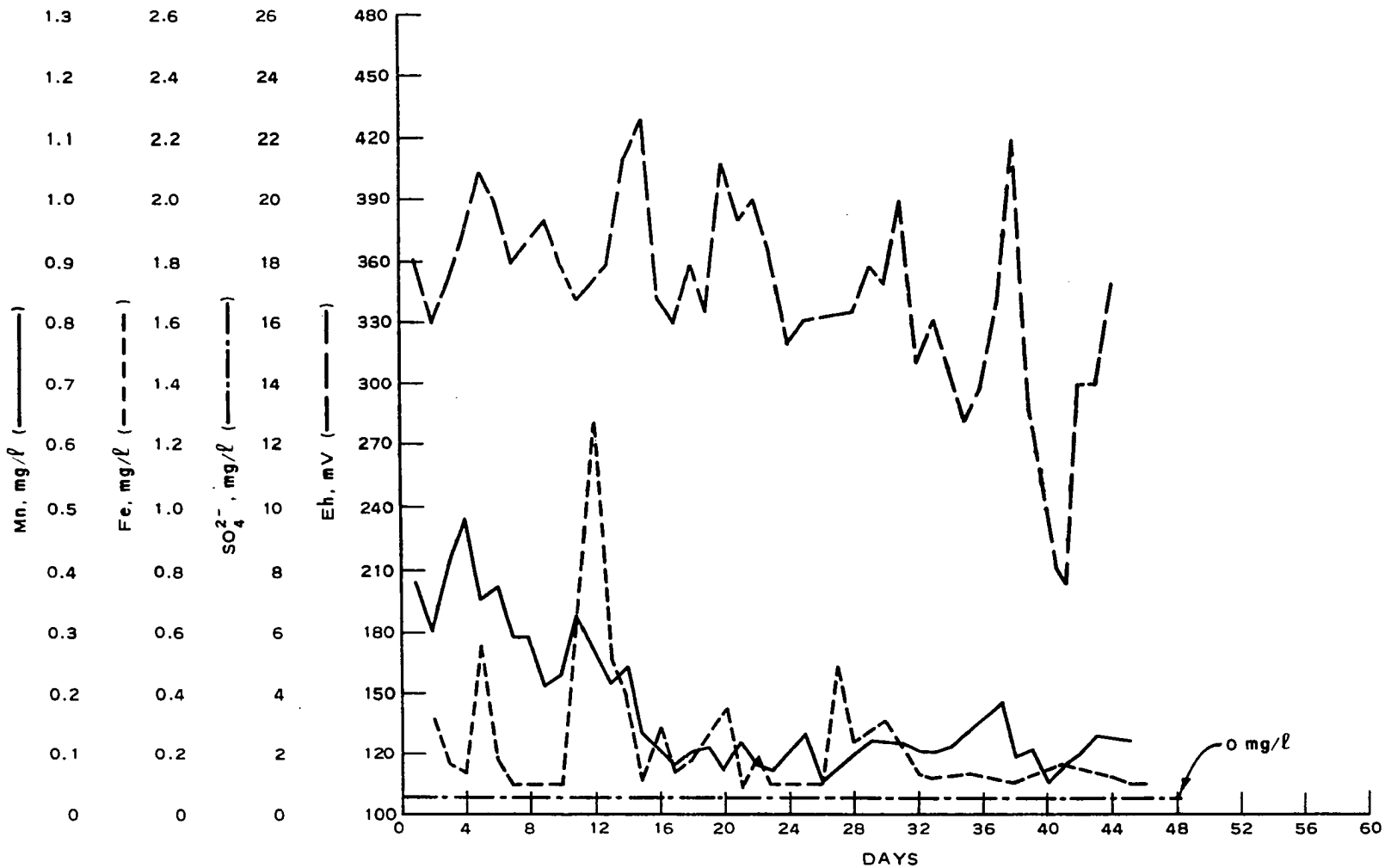


Figure 15. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 6 Effluent During Experimental Run 1

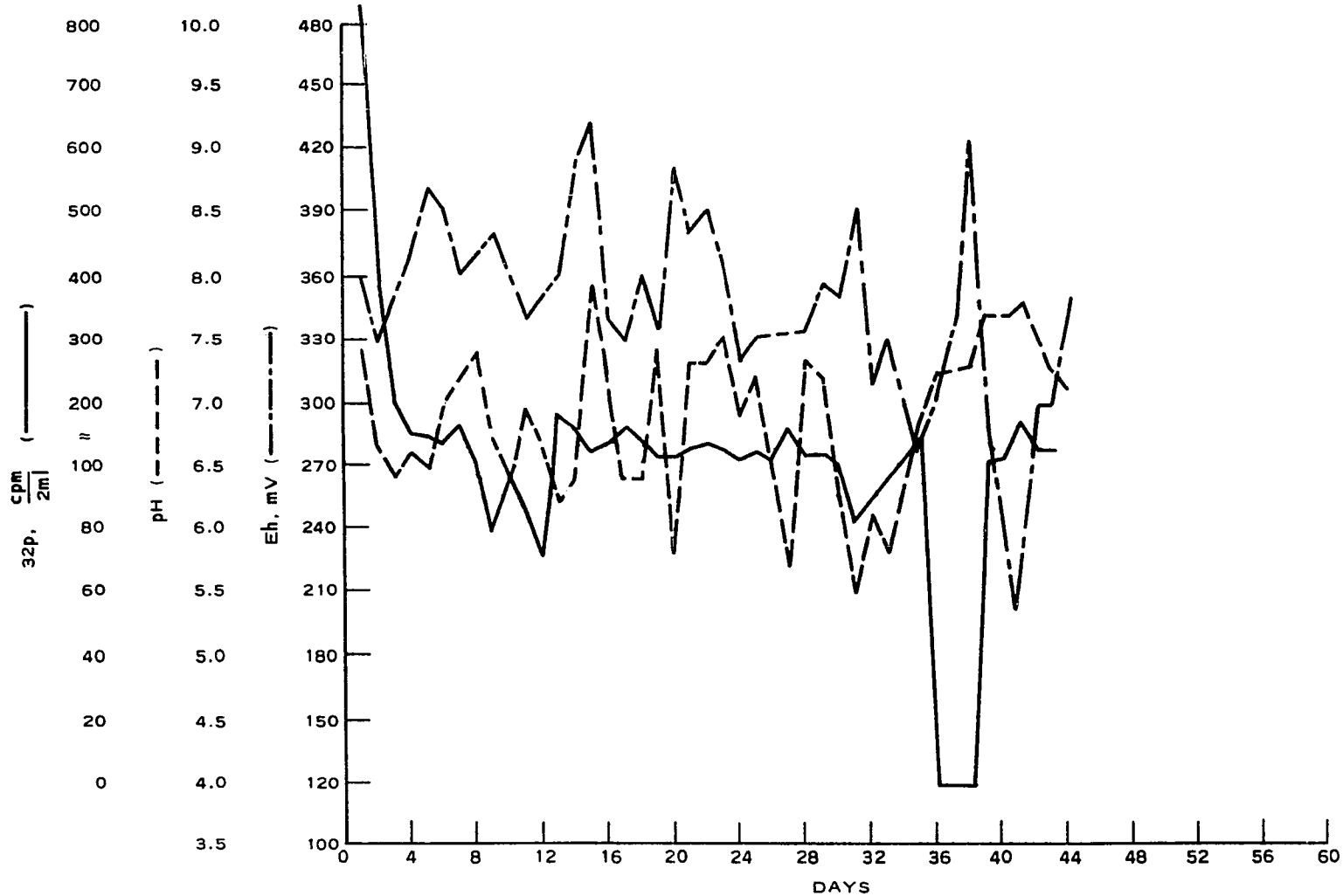


Figure 16. Comparison of ^{32}P, pH, and Eh for Column 6 Effluent During Experimental Run 1

indigenous heterotrophic organisms. Distilled water was passed through each cultivation chamber at 2 ml/hr with a 24-hour hydraulic retention time. On day 38, the air-water interface in the cultivation chamber of column 6 was replaced by a nitrogen-water interface. There was an air-water interface in the cultivation chamber of column 5 for the entire experimental run. The general decreasing trend for the effluent iron and manganese concentrations from both columns was observed from day 1 through day 8. Figure 13 shows that the effluent manganese concentration remained constant between days 2 and 4. Figure 15 shows that the effluent manganese concentration increased between days 2 and 4. Between days 3 and 5, the effluent iron concentration for column 5 increased as shown in Figure 13. A similar trend for column 6 also occurred (Figure 15). This trend indicates that the electrode potential was low enough to reduce some of the iron and manganese that either had adhered to the walls of the cultivation chambers or was at the water-sediment interface. It may also indicate that washout occurred as previously, even though turbidity was not evident. By comparing Figures 13 and 15, it can be seen that the effluent manganese concentration began to increase a day or two before the effluent iron concentration began to increase. This would be expected if the redox potential was the controlling factor, because the redox potential for the manganese cycle is higher than that for the iron cycle.

Another manganese and iron release from the sediment in columns 5 and 6 occurred between days 9 and 15. Figure 13 shows that a slight manganese release from the sediment may have occurred, from

about 0.13 mg/ℓ on day 8 to about 0.24 mg/ℓ on days 9 through 11; then the effluent manganese concentration decreased through day 15 to 0.13 mg/ℓ. Figure 15 shows that a very similar manganese release occurred from column 6 between days 9 and 15.

Next, a large iron release was observed in column 5 between days 10 and 15. During this time, the effluent iron concentration increased from 0.10 mg/ℓ on day 10 to 2.58 mg/ℓ on day 12. The same general trend occurred in column 6. On day 10, the effluent iron concentration was 0.10 mg/ℓ, and subsequently it increased to a maximum of 1.26 mg/ℓ by day 12 (Figure 15).

The large iron and manganese releases observed in columns 5 and 6 also occurred in columns 1 and 2. This strongly indicated that biologically mediated redox changes in the sediment were controlling these releases. As stated earlier, the releases of iron and manganese would be expected from this continuous-flow apparatus if the releases occurred at the sediment-water interface since the flow of water is over and up from the sediment. Once the affected iron and manganese in the sediment were washed out of the sediment, future releases of these metals from the sediment would be governed by other mechanisms, eg. diffusion, mixing, etc.

From days 15 through 46, the effluent iron and manganese concentrations remained fairly constant, indicating that very little iron and manganese was made available for further release. That is, relatively little iron and manganese passed from lower sediment to the sediment-water interface via diffusion and turbulence at the apparently low sediment redox potential.

Figures 13 and 15 also indicate that the electrode potential (Eh) as measured in the effluent was not at all representative of the sediment or the column water. Reasons similar to those stated for columns 1 and 2 also apply for columns 5 and 6. Apparently, the effluent water was reaerated between the cultivation chamber and the effluent chamber as it passed through the overflow tube. This seems to be validated by considering what happened to the effluent electrode potential of column 6 after day 38. Nitrogen gas was used to purge the air from the cultivation chamber and the effluent receptor. The result was a sharp decrease in the effluent electrode potential from 420 to 210 mV between days 38 and 41. The sharp rise from days 41 to 44 may simply reflect a relatively unstable redox potential at the sediment-water interface. This may have been due to the biological community being nutrient-limited; thus, the sediment-water interface could not maintain a stable community of heterotrophic organisms. Note also in Figures 5 and 15 that the electrode potential sharply increased between days 41 and 43. However, in column 1, the electrode potential remained low, whereas in column 6 the electrode potential increased. This would further indicate that the biological community stability was affected by the nutrient-free distilled water. Since the heterotrophic community is responsible for mediating the redox condition at the sediment-water interface, the electrode potential measurement would also reflect instability if a nutrient was limiting.

The sulfate concentrations in columns 5 and 6 were 0 mg/ℓ as shown in Figures 13 and 15. Since the distilled water did not

contain sulfate in measurable quantities, these concentrations were expected.

Figures 14 and 16 show the daily fluctuations of pH and ^{32}P in the effluent from columns 5 and 6, respectively. The typical rapid decrease or washout of ^{32}P occurred between about days 1 and 8 (Figure 14). From day 8 to day 12, negligible ^{32}P was detected in the effluent. Between days 13 and 30, except for day 24, a general release of ^{32}P from algae to the water occurred. The beginning of this ^{32}P release coincided with the release of iron (Figure 13). It would seem that the ^{32}P release was associated with a low redox condition in the water and surficial sediment of column 5. Since the iron release was rapid with respect to the sustained ^{32}P release, the bulk of the ^{32}P released was not associated with iron. The ^{32}P may have been caused by some other redox reaction or increased biological mediated release. From days 31 through 45, only two releases of ^{32}P occurred on days 37 and 43. In Figure 16, a similar washout of ^{32}P occurring in column 6 from days 1 through 4 is shown. From day 4 through day 43, except for days 36 through 38, a constant release of ^{32}P, i.e., 100 cpm/2 ml, occurred in this column.

It is quite evident that the ^{32}P release from columns 5 and 6 was significantly greater than that for any of the other previously discussed columns. A possible explanation could be that the heterotrophic community in columns 5 and 6 was not actively increasing in size and diversity because of a lack of certain nutrients which would normally be available to the sediment biota in lake water. Therefore, the phosphatase activity would continually break down the algal cell

and release ^{32}P to the water. If ^{32}P were not biologically utilized and/or physically sorbed, this sustained release would be a result of phosphatase degradation of organic particulate ^{32}P with little biota utilization of the available mineralized ^{32}P. If this is true, some other element besides phosphorus was probably limiting to the biota in the columns. The difference in the ^{32}P release from column 5 and column 6 may or may not be real. The quantity of ^{32}P released from both columns was fairly small, so differences may be overemphasized by the graphs.

The daily fluctuations in pH from both columns remained fairly small. In general, over approximately 45 days the pH was buffered between 6.5 and 7.5. This is generally similar to the effluent water quality of all of the columns previously discussed.

There were no visible physical changes in these columns for the duration of the run. That is, the algal residue remained green under the operating conditions.

Column 7

The data from column 7 are shown in Figures 17 and 18. The sediment in column 7 was sterile, and distilled water was passed through the column in an aerobic atmosphere. The slightly turbid effluent from this column contained suspended clay particulates from days 1 through 7. This resulted in a washout of iron and manganese through the first several days of operation similar to that observed for the previous columns. From day 8 through day 46, the effluent iron concentration was very low (<0.10 to 0.20 mg/ℓ). In fact, the

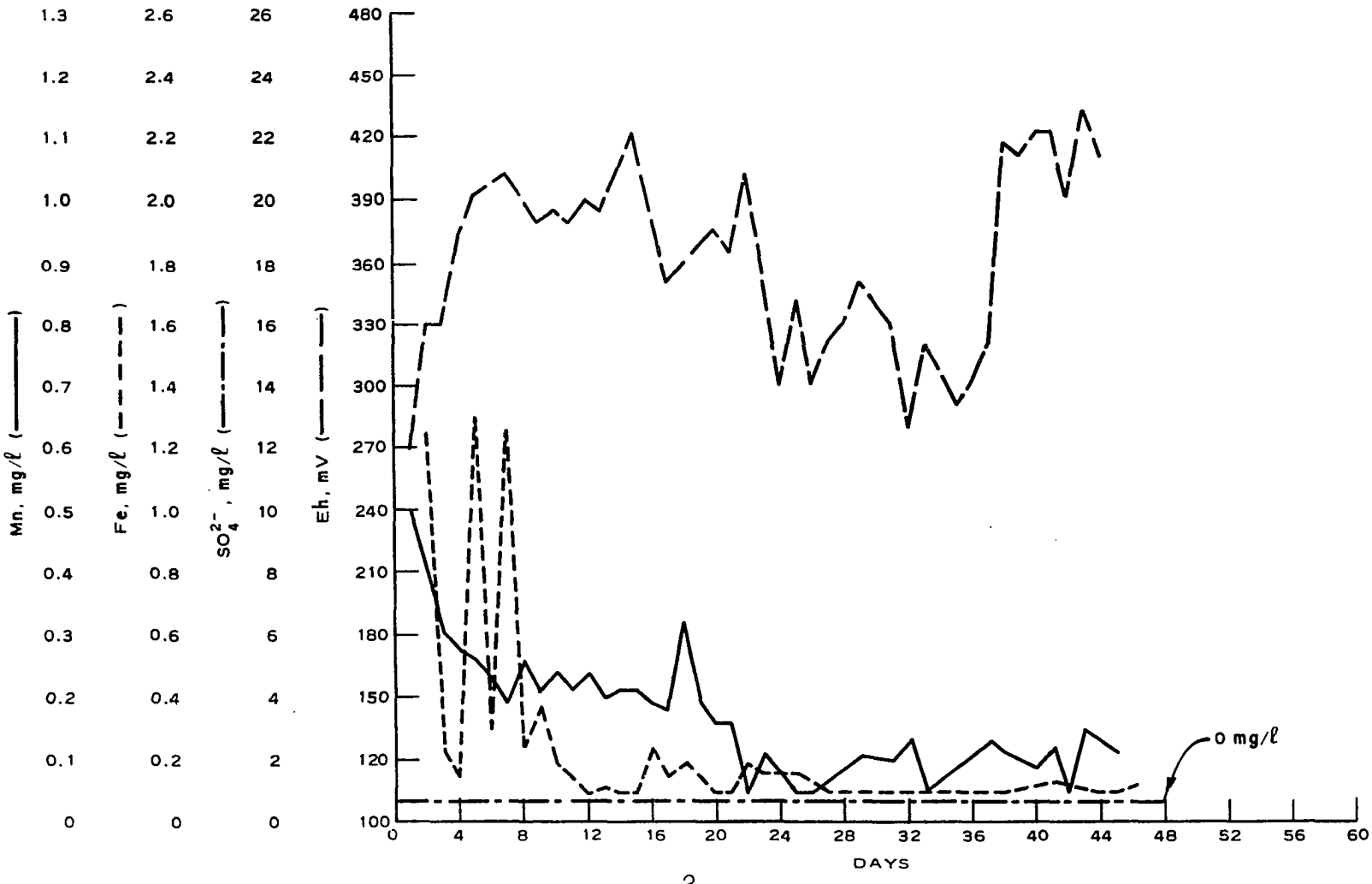


Figure 17. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 7 Effluent During Experimental Run 1

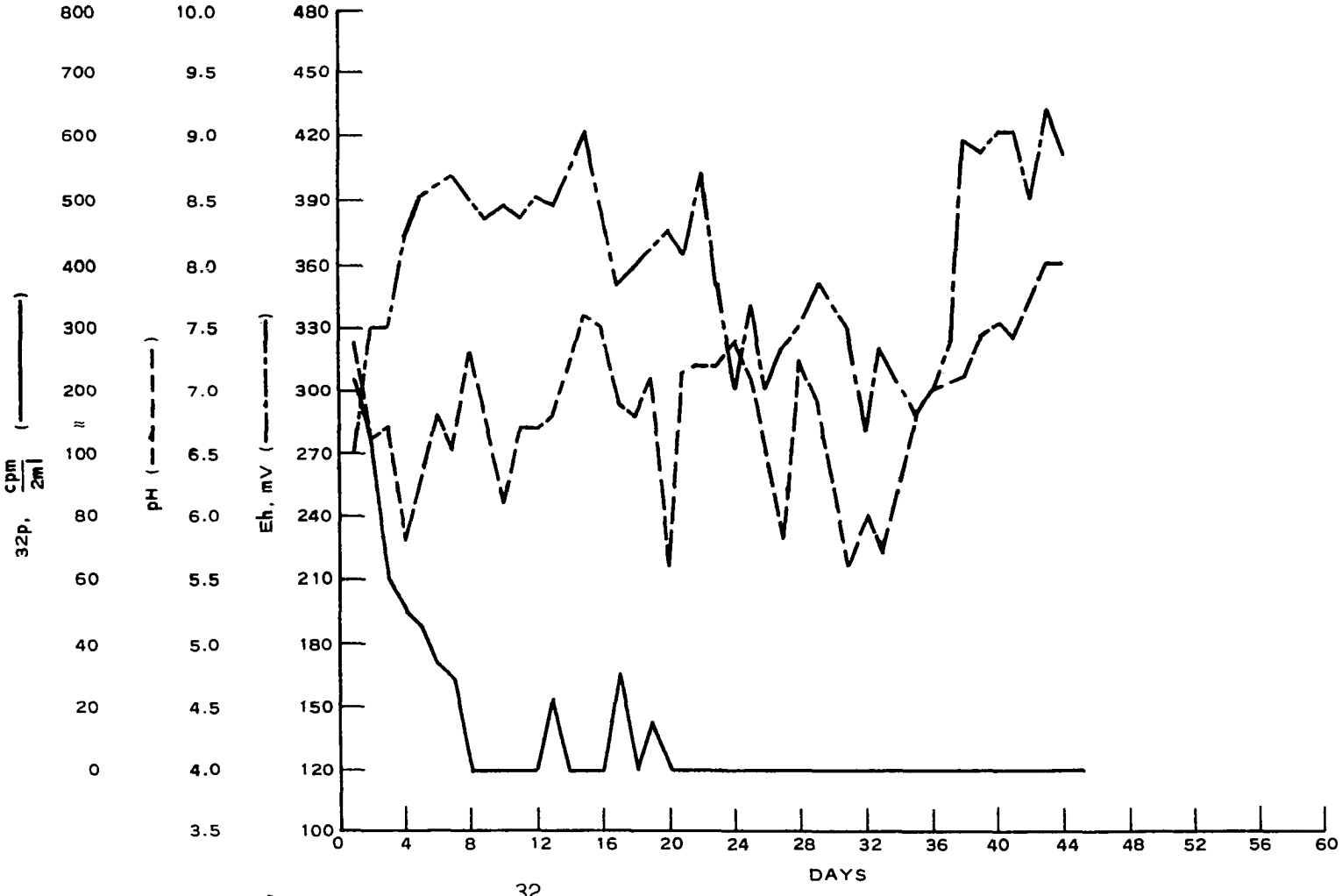


Figure 18. Comparison of ^{32}P, pH, and Eh for Column 7 Effluent During Experimental Run 1

effluent iron and manganese concentrations were very similar to those concentrations in the influent water. Note that, from days 7 through 21, the effluent manganese concentration remained constant at about 0.20 mg/ℓ. After day 21, the effluent manganese concentration dropped to about 0.10 mg/ℓ. The higher manganese concentration between days 7 and 21 may have been evidence of the fact that there were clay particles adhering to the walls of the cultivation chambers above the sediment-water interface as a result of autoclaving. The continual washing effect of the water flowing through the column resulted in the higher effluent manganese concentrations. This same trend occurred in columns 3 and 4 (Figures 9 and 11, respectively).

The electrode potential (Eh) of the effluent seemed to increase from 270 mV on day 1 to 407 mV on day 7. There was an apparently gradual decrease to about 300 mV on day 24. From day 24 through day 36, the electrode potential oscillated around 310 mV. After reconnecting a new supply of sterile, distilled water on day 37, the electrode potential increased to 410 mV by day 38 and remained constant through day 44. This increased oxidizing condition in the column was very similar to the electrode potential measured for the distilled water (see Appendix D). Again, the redox condition of the column effluent as measured by the electrode potential after only a 15-minute equilibration period should be interpreted in very general terms. The initial increase in the electrode potential from days 1 through 7 was possibly due to washing out the organic acids, etc., that had been released from the sediment and biota as a result of the autoclaving process.

There were also no apparent changes in the sulfate, pH, and ^{32}P that could be correlated with either the iron or manganese release pattern. The effluent sulfate concentration was 0 mg/ℓ from day 1 through day 48. This was the same as the sulfate concentration in the influent distilled water.

The effluent pH and ^{32}P concentrations are shown in Figure 18. After the expected washout of ^{32}P from day 1 through day 7, virtually background levels of ^{32}P were detected through day 45. On days 13, 17, and 19, only 20 to 25 cpm/2 ml were detected. These few releases seem to be anomalous with respect to the overall trend of effluent ^{32}P as indicated. Hence, there was no indication from these data that the algal particulate organic ^{32}P was released through an autodegradation process or by bacterial contamination of the cultivation chamber. These data indicate that an autodegradation process of algal organic matter is virtually nonexistent over a 45-day time period. Likewise, this system clearly indicates that this apparatus could be maintained for periods of greater than 45 days as a sterile system.

The effluent pH remained fairly constant between 6.0 and 7.5 for 41 days. Then it increased slightly to approximately 8.0 by day 43.

There were no visible physical changes in the sediment or algal residue of column 7 during the experimental run.

Experimental Run 2

The final portion of this experimental design attempted to more

closely simulate a natural environment above the sediment core and thus to study the degradation and subsequent release of ^{32}P to the overlying water. In the prior experimental run, very little ^{32}P was released to the overlying water when lake water was utilized. Likewise, the last run indicated that several factors may have prevented ^{32}P from being released. To investigate these factors in a reasonable time period, it was decided to forego analyzing sterile sediment controls. The information on the controls from the previous run indicated that the sterile sediment columns were not necessary. The ^{32}P release was negligible from the control columns of run 1.

The first factor considered was an increase in the specific activity of ^{32}P in the particulate organic cellular components of the algae. This was clearly indicated as being necessary based on the length of the first experimental run. Second, it was necessary to operate the columns with an inert atmosphere. Third, it was necessary to use lake water with an increased concentration of phosphorus in order to better evaluate whether or not phosphorus could have been somewhat limiting to the sediment community in the columns of the previous experimental run. If the phosphorus were limiting, this could be the reason why the phosphorus was not released to the overlying water. Since distilled water is not at all similar to the water in a natural system, lake water (with its ambient nutrient concentration) was used to flow through all eight columns continuously. A 0.5-mg/ℓ quantity of unlabelled phosphorus as KH_2PO_4 was added to the lake water supplying columns 2, 4, 6, and 8. This would help determine if the ambient nutrient levels in the lake water had an

effect on the release of ^{32}P and/or any other stated chemical parameters. This water was used to feed columns 2, 4, 6, and 8. Lake water without additional phosphorus was used in columns 1, 3, 5, and 7. Fourth, it was necessary to determine if the ^{32}P would be released by decreasing the HRT. It was suspected that the flow rate and volume (2 ml/hour, 24-hour HRT) relationship may have allowed the mineralized ^{32}P to precipitate out of solution or onto the column's wall prior to passing out the overflow tube. Therefore, on several columns, the flow rate was increased, and the volume of water overlying the sediment core was reduced. Finally, it was necessary to determine if results would be obtained similar to those in experimental run 1. This was accomplished by continuing columns 1 and 2. These columns were reinoculated with more labelled algae and were purged with nitrogen gas. Column's 3 through 8 were started in a manner similar to that for columns 1 through 4 of experimental run 1; however, they were purged with nitrogen gas from day 1.

Columns 1 and 3

The pertinent data from columns 1 and 3 are shown in Figures 19, 20 and 21, 22, respectively. Column 1 was continued from experimental run 1, whereas column 3 was restarted with a fresh sediment core. The initial washout of manganese and iron, which was observed from the columns during the first experimental run and from column 3 (Figure 21) of this experimental run, did not occur in column 1 (Figure 19). This was expected since the sediment in column 1 was not disturbed upon addition of fresh, ^{32}P-labelled algal residue. Also, column 1 had already

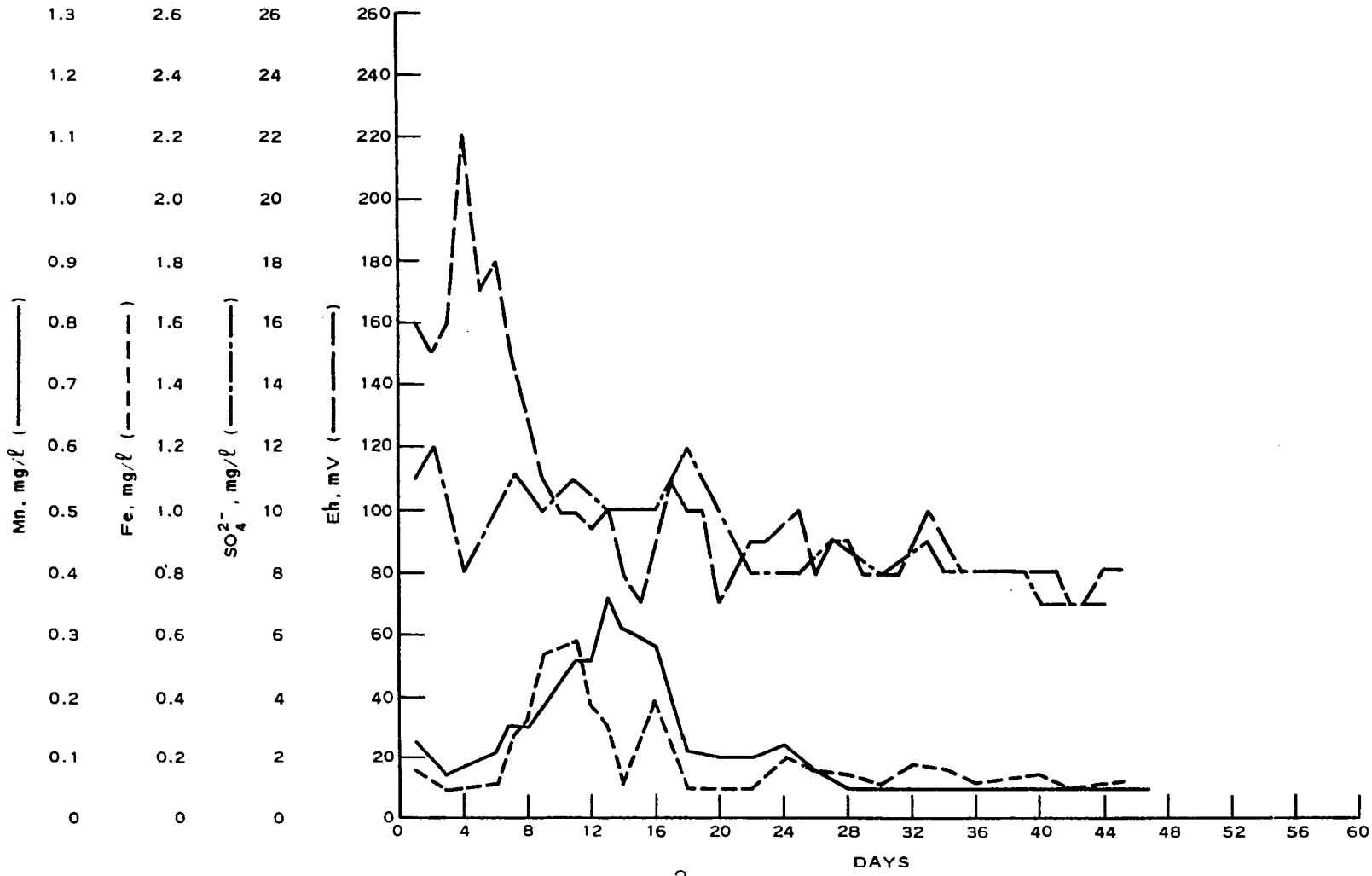


Figure 19. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 1 Effluent During Experimental Run 2

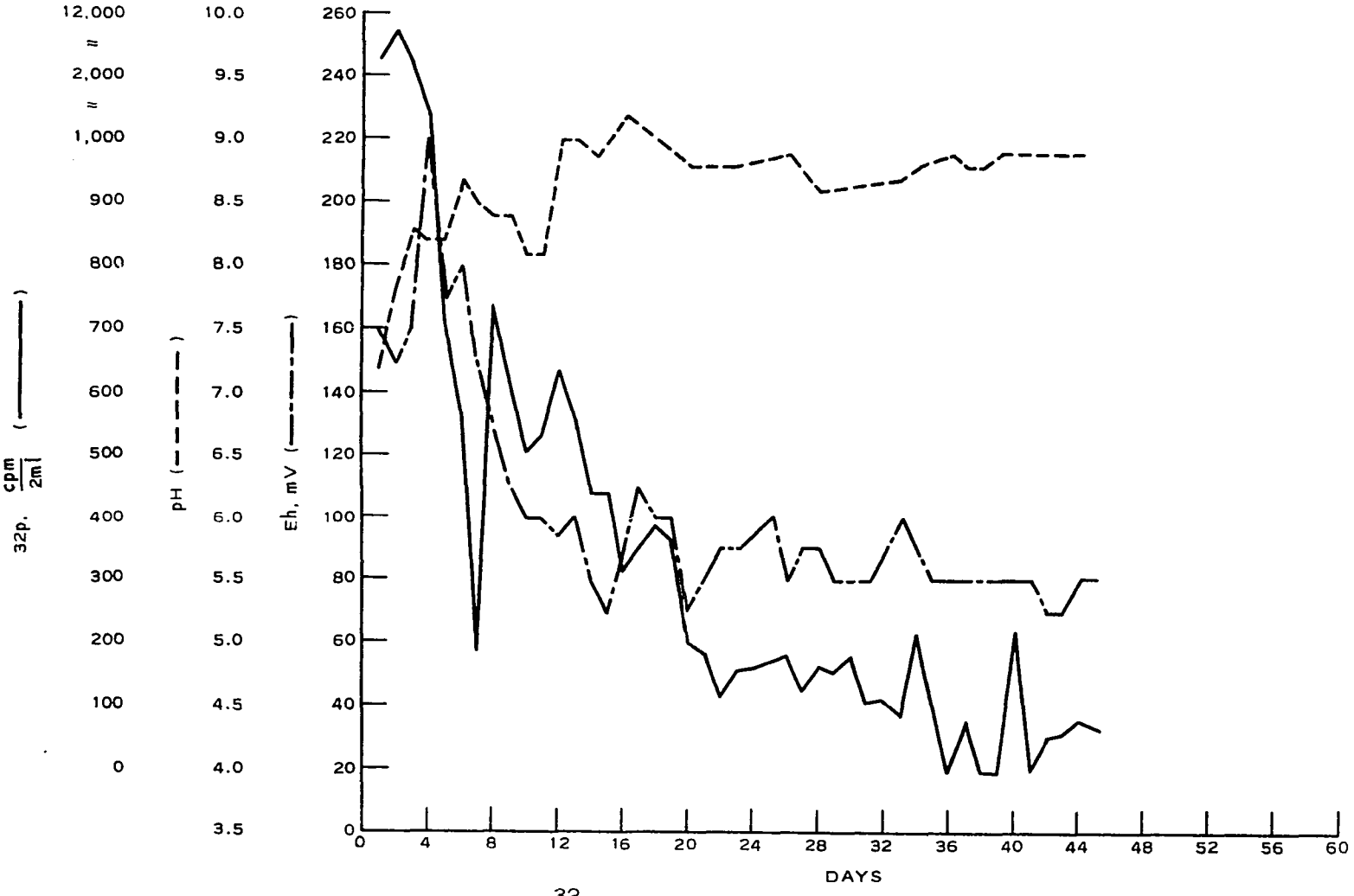


Figure 20. Comparison of ^{32}P, pH, and Eh for Column 1 Effluent During Experimental Run 2

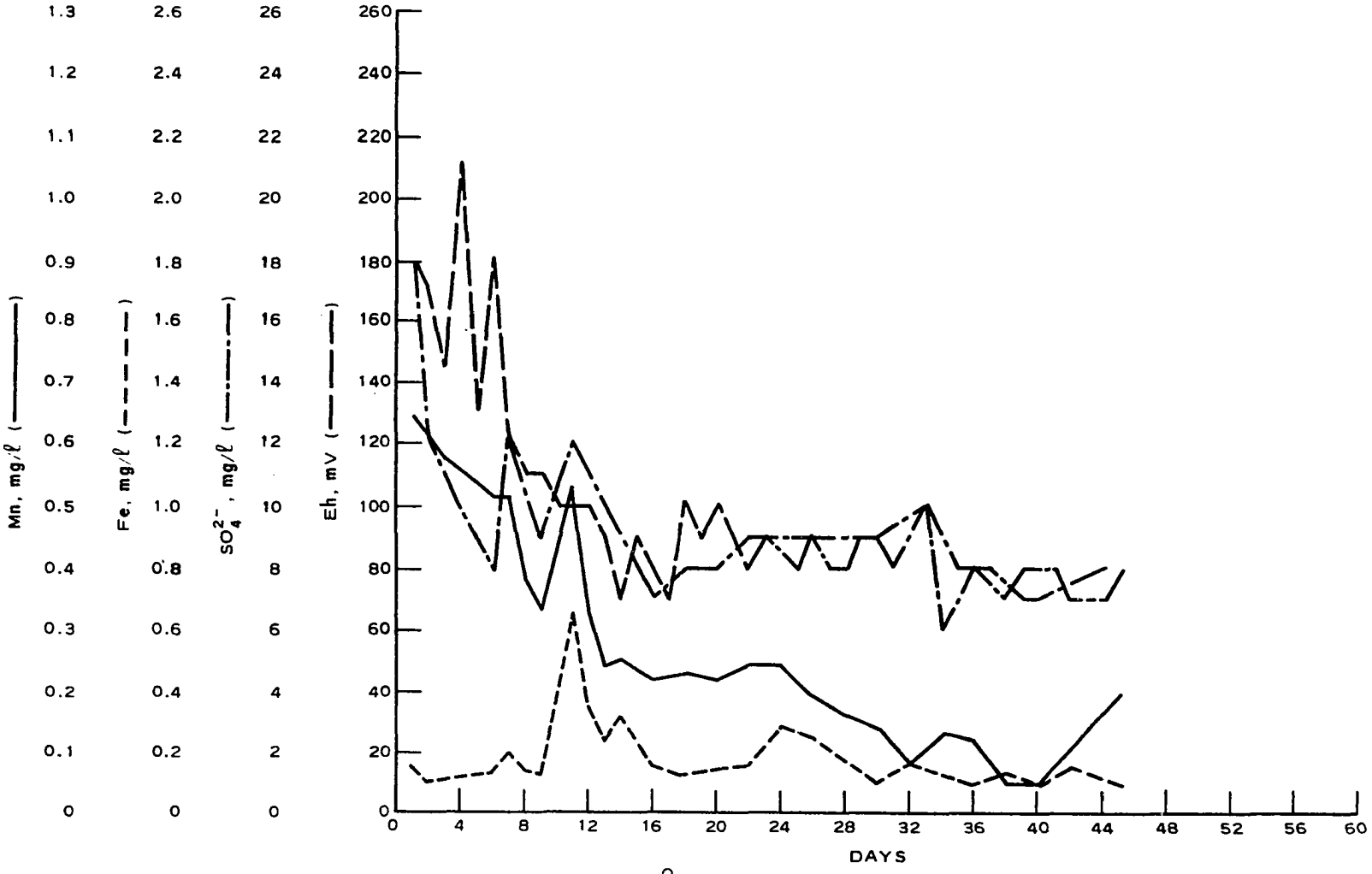


Figure 21. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 3 Effluent During Experimental Run 2

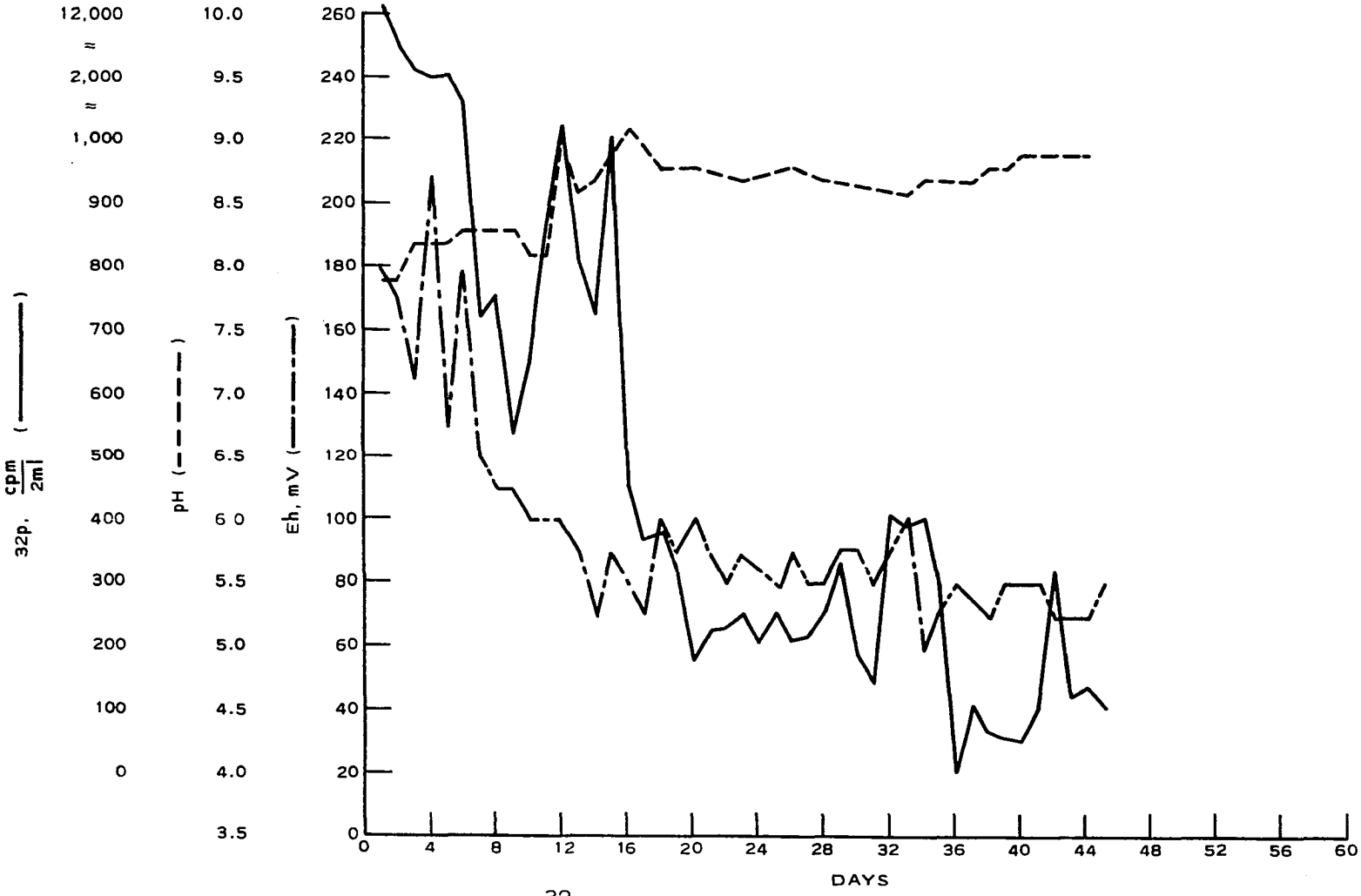


Figure 22. Comparison of ^{32}P, pH, and Eh for Column 3 Effluent During Experimental Run 2

washed out the manganese and iron associated with the preparation of the core at the beginning of the first experimental run.

Figure 19 shows that from days 3 through 18, there was a gradual increase in the release of manganese (Mn) from column 1. A peak release occurred on or about day 13. At this time, 0.35 mg/ℓ of manganese was recorded in the effluent of column 1. From days 18 through 45, the effluent manganese concentration decreased to less than 0.05 mg/ℓ. Also, from about day 6 through day 18, a noticeable increase in the effluent iron concentration occurred. From days 18 through 45, the effluent iron concentration was stable between 0.10 and 0.20 mg/ℓ. About the same time that the effluent manganese and iron concentrations were increasing, black coloration began to be noticed in the fresh algal residue layer placed initially on the older black algal residue.

From column 3, a release of iron and manganese occurred similar to that observed in column 1 (Figure 5) shortly after that column was set up and was operating. Another release of manganese occurred between day 9 and day 13 with a maximum effluent manganese concentration of 0.53 mg/ℓ on day 11. From day 13 through day 40, a gradual decreasing manganese release occurred until days 38 through 40 when the effluent manganese concentration was at a minimum (<0.05 mg/ℓ). From days 40 to 45, a slight increase to 0.20 mg/ℓ Mn was observed. This increase may have been due to the diffusion of manganese from the lower sediment to the sediment-water interface. Also from Figure 21, it can be seen that a similar iron release occurred paralleling the release of manganese from column 3. The characteristic washout of

iron observed during the first few days of the last experimental run did not occur. The columns were set up much more carefully, and turbidity was thus minimized. From days 1 through 9, the effluent iron concentration was less than 0.10 to 0.20 mg/ℓ. Between days 9 and 16, a significant iron release occurred with a maximum effluent iron concentration of 0.68 mg/ℓ measured on day 11. From day 18 through day 45, the effluent iron concentration remained between 0.10 and 0.20 mg/ℓ. This was slightly more than the iron concentration of the influent water media (see Appendix D).

From Figures 19 and 21, it can be seen that the release patterns of iron and manganese occurred at about the same time in both columns. Likewise, the releases occurred when the effluent electrode potential was between 80 and 100 mV. During this same time, the labelled algal residue in both columns began to turn black. It could thus be surmised that the redox condition at the water-sediment interface was probably much lower than that indicated in the columns' effluent.

The effluent sulfate concentration of column 1 on day 1 was much lower than that of the influent water (Appendix D). The effluent sulfate concentration of column 3 dropped from about 18 mg/ℓ on day 1 to a minimum of about 8 mg/ℓ by day 6 and remained fairly constant throughout the experimental run. This was further evidence of the fact that the redox potential in columns 1 and 3 at the sediment-water interface was much lower than what the electrode potentials were measuring in the effluent of these columns. Based on the effluent sulfate concentration, the electrode potential, and the black coloration, it may be hypothesized that the black coloration was caused by reduced sulfur

compounds, e.g., hydrogen sulfide and ferrous sulfide. Evidence from the data obtained at the conclusion of this run and discussed in Chapter V support this conclusion. The electrode potential for the first several days in column 1 (Figure 19) reflected what it had been at the conclusion of the first run, i.e., 160 to 200 mV. Upon addition of the algal residue, a further decrease in the electrode potential was noted after the fifth day of operation. From 220 mV on day 4, the electrode potential decreased to approximately 80 mV by day 15. The electrode potential generally remained constant for the remainder of experimental run 2. A very similar pattern occurred in column 3 as shown in Figure 21. Thus, it would appear that in the described manner of operation of these columns approximately 4 or 5 days elapse before a response is noted by the sediment heterotrophic community on the substrate of interest.

On day 1, the electrode potential (Eh) of column 3 was much lower than that of the influent water. This was expected, since the flow of water through the column was not started until 12 hours later so that most of the initial turbidity, etc., could be settled out prior to the experiment. During this 12-hour period, the electrode potential was decreased by the heterotrophic community.

The release of ^{32}P and the pH response of columns 1 and 3 are shown in Figures 20 and 22, respectively. From days 1 through 7, the characteristic washout of ^{32}P was observed in column 1 (Figure 20). From day 7 through day 20, the effluent ^{32}P increased from about 200 cpm/2 ml on day 7 to 750 cpm/2 ml on day 8. A decreasing release of ^{32}P from the algal residue was observed from day 8 through day 36

when only background levels of ^{32}P were counted. Thus, for the duration of the second experimental run, less and less ^{32}P was released to the overlying water. This followed the pattern of column 1 during the first run. The quantity of ^{32}P released during this experimental run was a very small percentage (estimated <10 percent) of the total ^{32}P added to the column initially.

A very similar release pattern was noted for column 3 as shown in Figure 22. There was a rapid washout from day 1 through day 9, i.e., 13,000 to 550 cpm/2 ml. As stated earlier, this washout was expected. From days 9 through 15, the effluent ^{32}P increased to greater than 1000 cpm/2 ml by day 12. The effluent ^{32}P count rate decreased to about 730 cpm/2 ml on day 14; then, it increased to greater than 1000 cpm/2 ml by day 15. It would appear that the release may have been associated with the iron and manganese release or a result of mineralization and subsequent leaching as particulate ^{32}P. Following this increased release was a rapidly declining release to about 200 cpm/2 ml by day 20. From day 20 to day 35, a steady but low ^{32}P count of approximately 200 cpm/2 ml was detected. From day 35 to day 45, only a constant 100 to 200 cpm/2 ml was detected in the effluent. Again, the quantity of ^{32}P accounted for in the effluent represented only a small percentage (estimated <10 percent) of the total ^{32}P in the column.

The pH responses of both columns 1 and 3 were very similar to the response of column 1 in the first run after it has been purged with nitrogen gas. There was a gradual increase to a pH of about 8.7;

then, it remained fairly constant for the duration of the run.

As stated earlier, the physical changes observed in both of the columns were confined primarily to the formation of the black coloration in the algal residue. This change began to occur about 5 days after the start of column 1 and about 8 days after the start of column 3. By the end of day 15, the algal residue was almost entirely black.

Column 1 was utilized in the experimental apparatus for 105 days. Realizing the biological community in the sediment of this column may have been drastically altered in relation to the diversity and concentration of the original heterotrophic community, the response of the column was still very reproducible from runs 1 and 2 with respect to the parameters measured.

Columns 2 and 4

The data from columns 2 and 4 are shown in Figures 23, 24 and 25, 26, respectively. The lake water being passed through these two columns contained additional phosphorus (0.50 mg P/ℓ) as KH_2PO_4. Otherwise, they were operated like columns 1 and 3. The primary objective of the tests with these two columns was to gain some insight as to whether or not phosphorus was limiting to the sediment community. These two columns would also help to better understand the effects of ambient phosphorus levels in lake water on the accessibility of sediment phosphorus to the overlying water.

In comparing Figures 23 and 25, which represent columns 2 and 4, respectively, it was readily seen that both columns yielded effluents

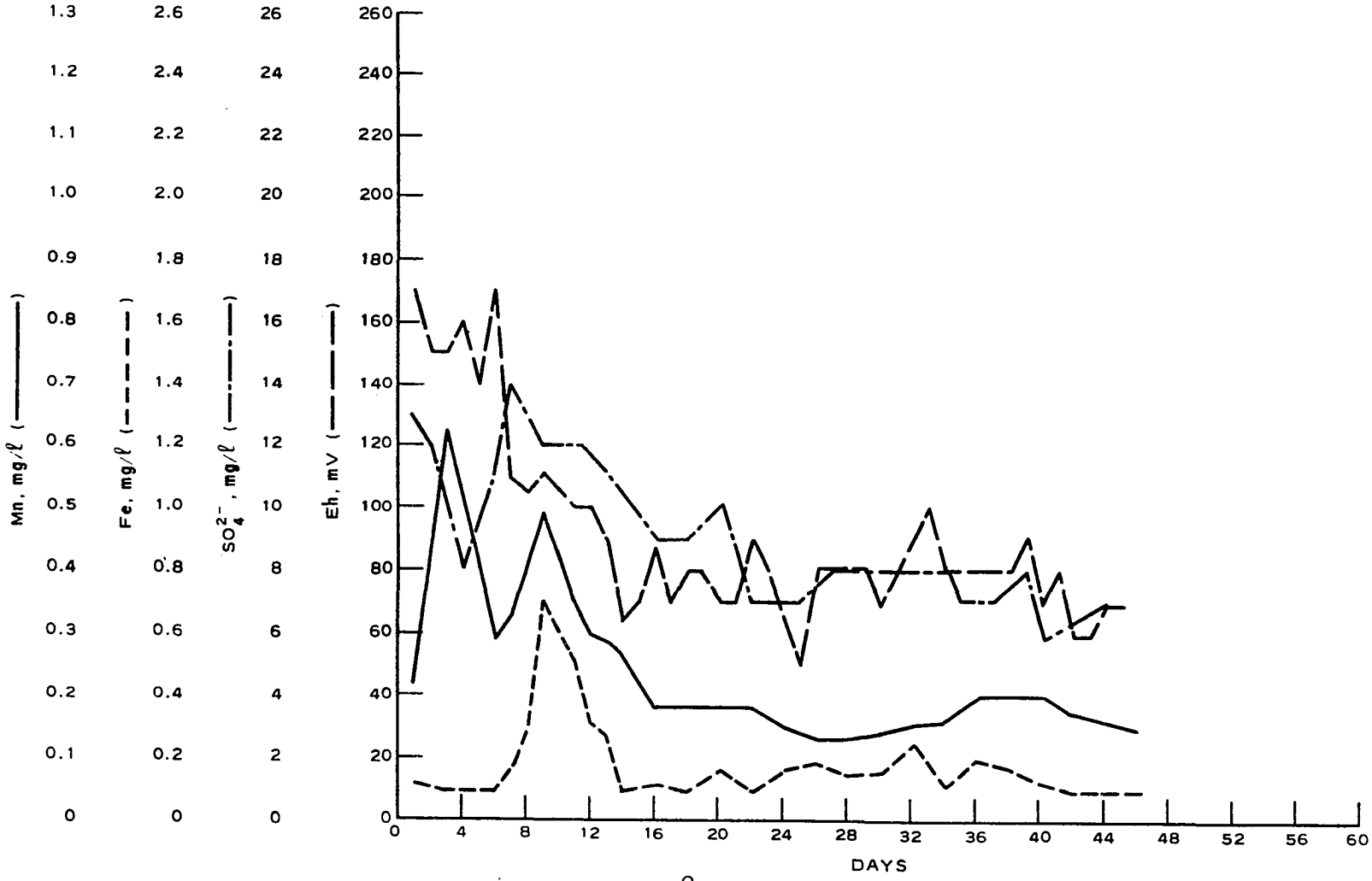


Figure 23. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 2 Effluent During Experimental Run 2

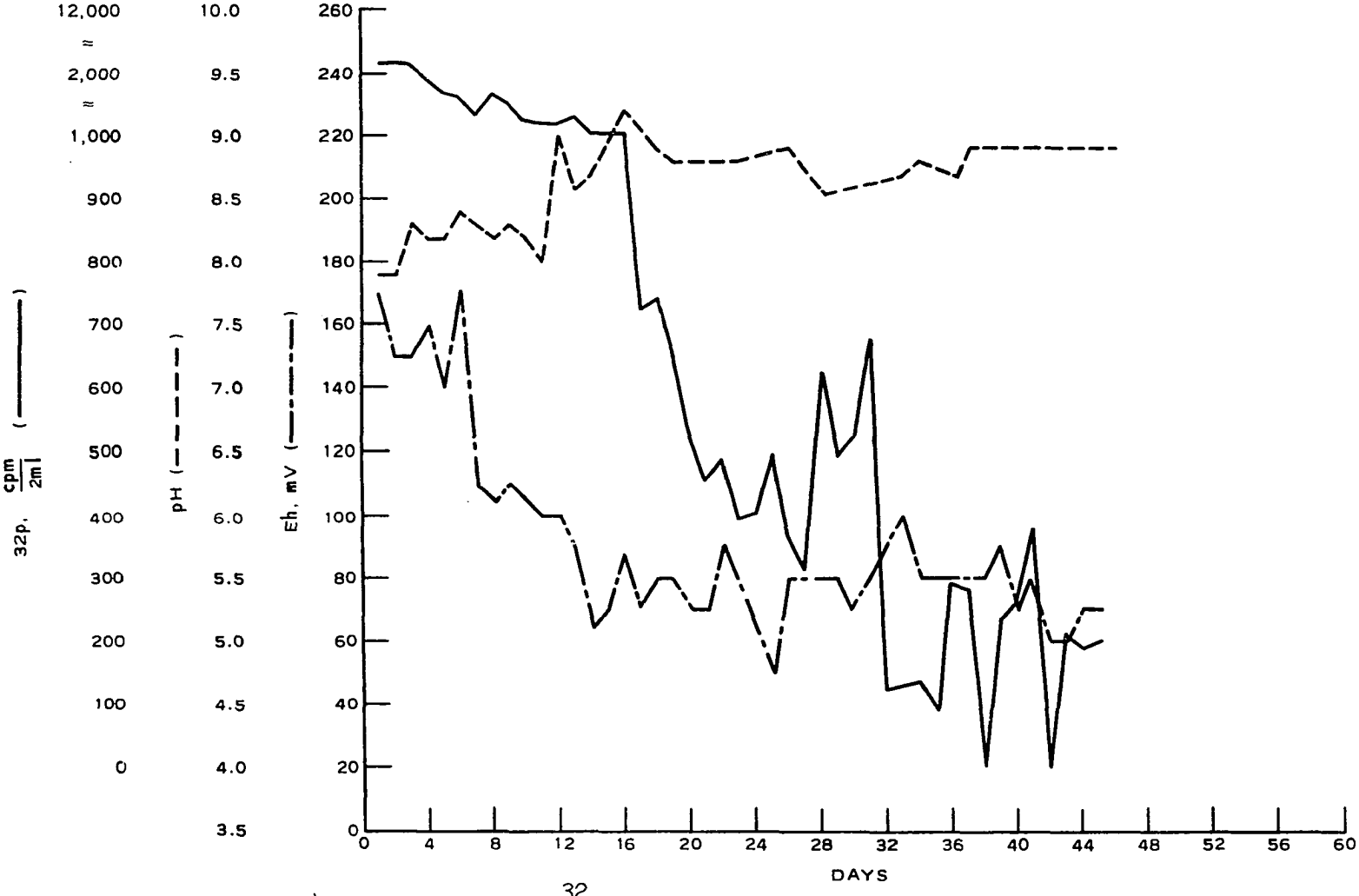


Figure 24. Comparison of ^{32}P, pH, and Eh for Column 2 Effluent During Experimental Run 2

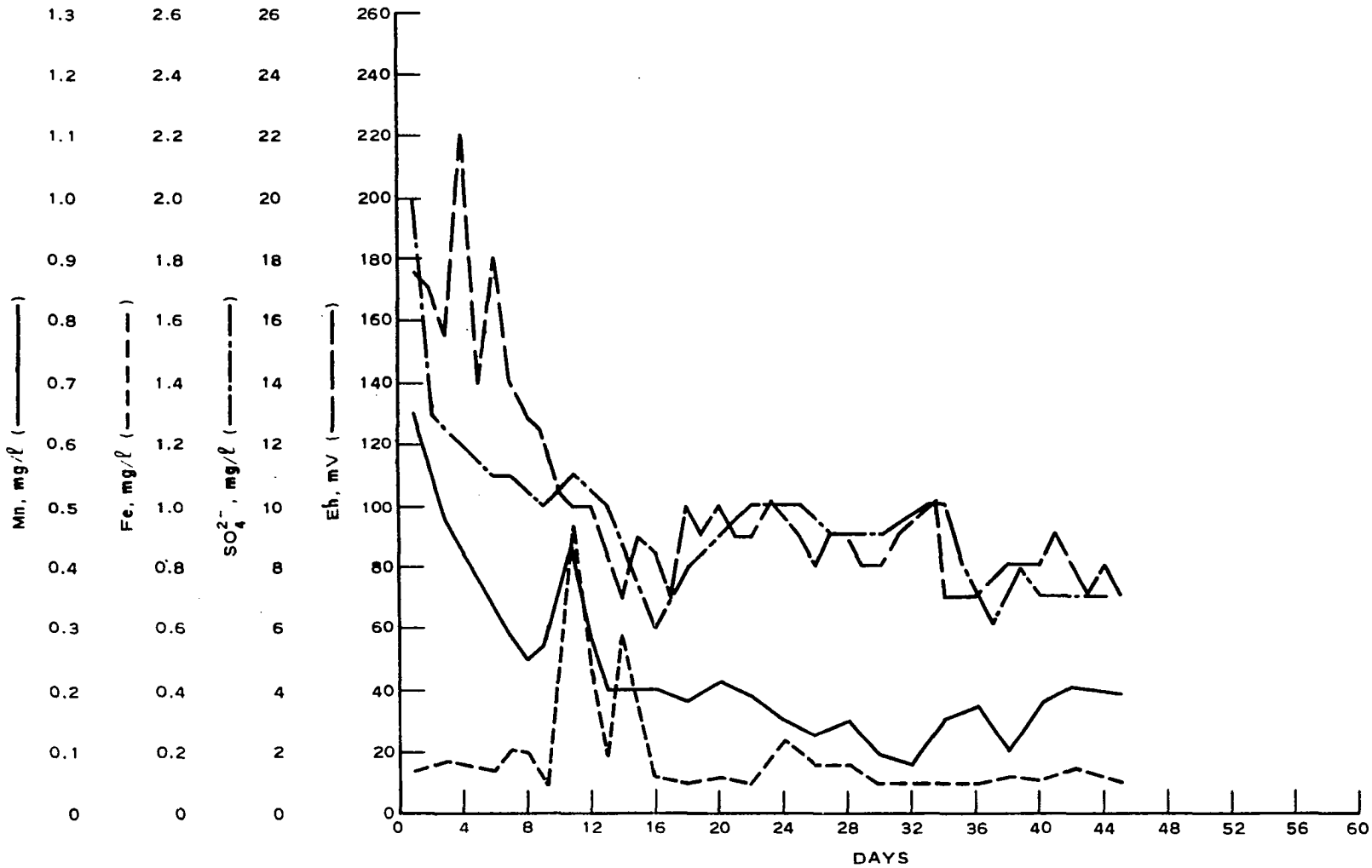


Figure 25. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 4 Effluent During Experimental Run 2

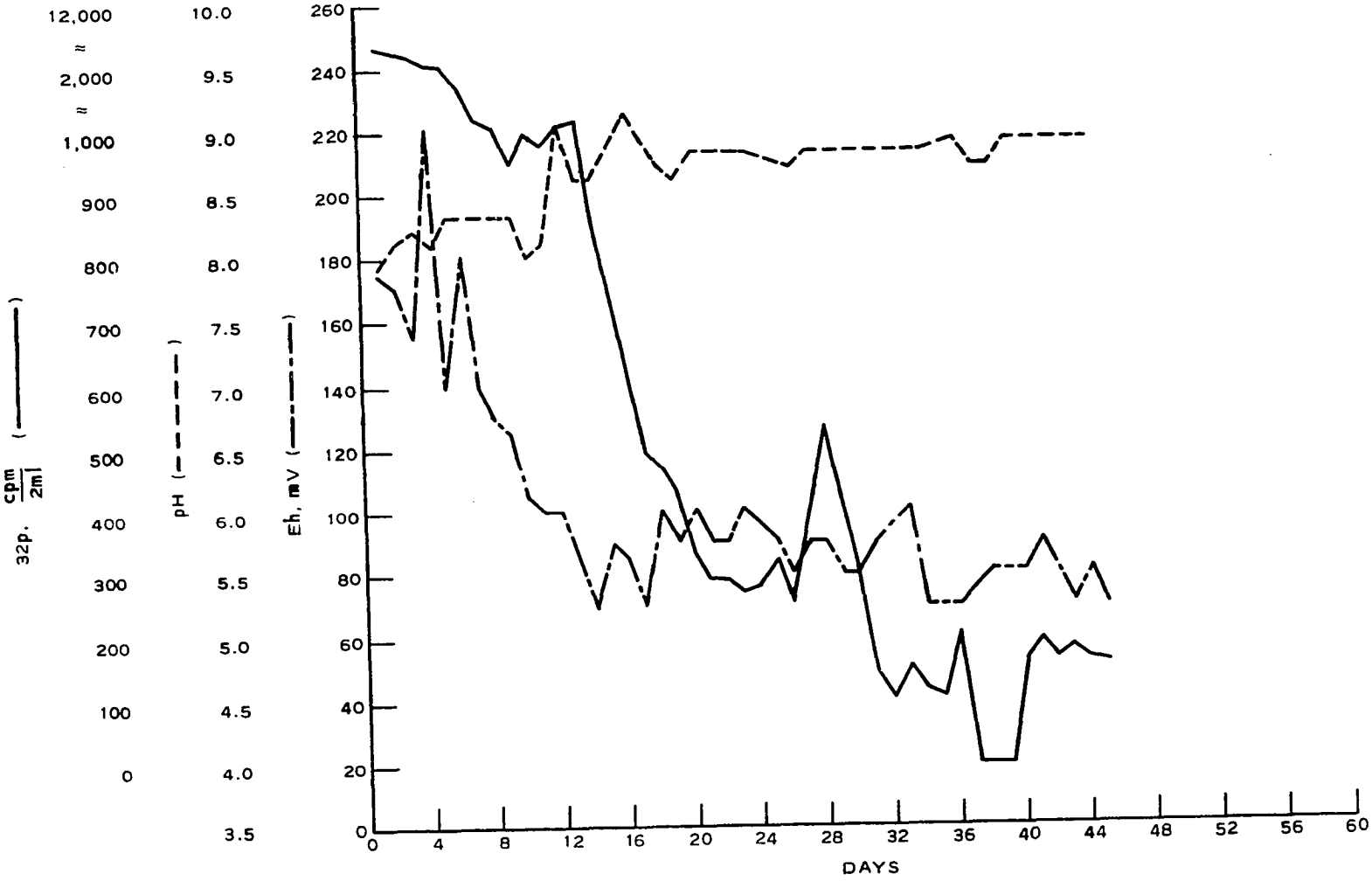


Figure 26. Comparison of ^{32}P, pH, and Eh for Column 4 Effluent During Experimental Run 2

with very similar water quality with respect to the parameters measured. Column 2 had been continued from the first experimental run. More ^{32}P-labelled algae were added, and nitrogen gas was used to purge the system in this column. Column 4 was started with a new sediment core for run 2.

As shown in Figure 23, there was an apparent release of manganese from column 2 between day 1 and day 5. During this time, the manganese concentration increased from 0.22 mg/ℓ on day 1 to about 0.62 mg/ℓ on day 3. By day 6, the effluent manganese concentration was down to 0.30 mg/ℓ. Then, from day 6 through day 12, the manganese concentration increased to a maximum of 0.50 mg/ℓ on day 9. A rapid decrease in the effluent manganese concentration followed through day 16 at which time 0.18 mg/ℓ was detected. From day 16 through day 46, the effluent manganese concentration remained constant between 0.15 and 0.20 mg/ℓ.

An analogous manganese release was observed in the effluent from column 4 as shown in Figure 25. Following the expected washout of manganese from days 1 through 8, a slight increase in effluent concentration occurred between day 8 and day 13. During this time, the manganese concentration increased from 0.30 mg/ℓ to about 0.44 mg/ℓ on day 11; then, it decreased to 0.20 mg/ℓ by day 13. From days 13 through 22, the effluent manganese concentration remained at 0.20 mg/ℓ. After day 22, the manganese concentration decreased to about 0.07 mg/ℓ on day 32. From day 32 through day 45, the manganese concentration remained fairly constant between 0.10 and 0.20 mg/ℓ. This was very

similar to the response of the influent lake water as shown in Appendix D.

Since both manganese and iron respond similarly to changing redox conditions, a similar pattern of iron release in both columns was expected. From Figure 23, it can be seen that the first 6 days of operation yielded less than 0.10 mg/ℓ of iron in the effluent from column 2. An increased iron release from the column occurred between days 7 and 13 with a maximum effluent iron concentration of 0.70 mg/ℓ occurring on day 9. From day 14 through day 46, the effluent iron concentration remained constant between 0.10 and 0.20 mg/ℓ.

In column 4, the iron release did not occur until day 9 and lasted through day 16. The maximum effluent iron concentration was 0.90 mg/ℓ on day 11. The double peaks on days 11 and 14 probably represent the same period of release in the column. From day 16 through day 45, the effluent iron concentration remained constant between 0.10 mg/ℓ and about 0.20 mg/ℓ.

The sulfate concentration in the effluent from columns 2 and 4 decreased to about the same concentration after day 13. From day 1 through day 4, a decreasing sulfate concentration was noted in the effluent of column 2. Between days 4 and 16, an increase in the effluent sulfate concentration was measured. The sulfate concentration increased from about 8 mg/ℓ on day 4 to a maximum of 14 mg/ℓ on day 7; the sulfate concentration then decreased to 9 mg/ℓ by day 16. This apparent release over the same time period that iron and manganese were being released may have been a result of an unstable redox condition in the cultivation chamber.

The sulfate concentration in the effluent of column 4 steadily declined from 20 mg/ℓ on day 1 to about 6 mg/ℓ on day 16. From day 16 through day 45, the effluent sulfate concentration ranged between about 6 and 10 mg/ℓ. Slight fluctuations were probably attributable to experimental variation in analysis or slight interferences. The fluctuations, however, were not significant.

The spread of sulfate concentrations in both columns would indicate that the redox condition at the sediment-water interface may have been fluctuating above and below the redox potential in which sulfates are reduced, i.e., less than -150 mV. The electrode potentials of the sediment after dismantling were much lower than those indicated by the effluent electrode potentials during the experimental run (Table 7).

The electrode potential of the effluents from both column 2 and column 4 steadily declined to between 80 and 100 mV by days 12 and 13, respectively. Generally, the electrode potential from column 2 effluent remained fairly constant between 70 and 80 mV after day 13 to the conclusion of the experimental run. A similar pattern existed for column 4; however, a slightly higher constant electrode potential of about 80 to 90 mV was measured.

The nitrogen atmosphere enabled a much lower effluent electrode potential to be determined. However, a much lower redox condition would have existed in the column because the effluent sulfate concentration was so low. This indicates that sulfates were being converted to sulfides.

Figures 24 and 26 show the ^{32}P and pH of the effluent from

TABLE 7

PROFILE OF THE SEDIMENT ELECTRODE POTENTIAL FROM COLUMNS 1-8

Column	Layer	H_2S Odor	Eh at 15 minutes
1	0-1 cm	yes	-100 mV
	1-2 cm*	no	0 mV
	2-3 cm*	no	+30 mV
2	0-1 cm	yes	-125 mV
	1-2 cm	no	0 mV
	2-3 cm	no	0 mV
3	0-1 cm	yes	-120 mV
	1-2 cm	no	0 mV
	2-3 cm	no	0 mV
4	0-1 cm	yes	-100 mV
	1-2 cm	no	0 mV
	2-3 cm	no	+20 mV
5	0-1 cm	yes	-150 mV
	1-2 cm	no	0 mV
	2-3 cm	no	+20 mV
6	0-1 cm	yes	-100 mV
	1-2 cm	no	0 mV
	2-3 cm	no	0 mV
7	0-1 cm	yes	-100 mV
	1-2 cm	no	0 mV
	2-3 cm	no	0 mV
8	0-1 cm	yes	-115 mV
	1-2 cm	no	0 mV
	2-3 cm	no	+10 mV

* A small amount of ion-free water was added to allow for these layers to be removed by a syringe.

columns 2 and 4, respectively. The rapid washout of ^{32}P, as observed in previous columns, was not observed in columns 2 or 4. Through day 16, the ^{32}P counted in the effluent of column 2 was greater than 1000 cpm/2 ml. After day 16, a rapid decrease in the counting rate occurred through day 27 when about 300 cpm/2 ml was noted. Between days 27 and 31, the ^{32}P activity in the effluent increased to a maximum of 600 cpm/2 ml on days 28 through 31; then, it decreased to between 100 and 300 cpm/2 ml for the remainder of this run.

A similar pattern was observed in column 4. The effluent ^{32}P activity was around 1000 cpm/2 ml through day 13. A rapid decline in the ^{32}P counting rate was observed through day 21. From days 21 through 26, the ^{32}P counting rate was about 300 cpm/2 ml. Between days 26 and 30, an apparent ^{32}P release occurred. The maximum ^{32}P activity was on day 28 at about 600 cpm/2 ml. From day 28 through day 32, the effluent ^{32}P activity decreased to approximately 100 cpm/2 ml. The effluent ^{32}P activity remained constant for the duration of the run.

Both columns' pH measurements responded in a manner similar to those of columns 1 and 3. The pH of column 2 increased to about 8.7 by day 13. The same trend also occurred in column 4. From day 13 to the completion of the run, the pH remained constant at about 8.7.

The main physical changes which occurred in these two columns were confined to the change in color of the algal residue from green to black. This color change started around day 8 in both columns and was completed on or about day 16.

From these results, it would seem that the ambient phosphorus

concentration in the overlying water did affect the phosphorus release from the sediment to some degree. Since the ^{32}P activity detected in the effluent represented a small fraction (estimated <10 percent) of the total ^{32}P in the column, isotopic exchange could explain the slightly higher effluent ^{32}P count in these columns.

Column 2 was continued from the first experimental run; consequently, it was active for over 100 days total. This column, when reinoculated with labelled algae for experimental run 2 and purged with nitrogen gas, responded very similarly to the measured water quality parameters of column 4.

Columns 5 and 7

Figures 27, 28 and 29, 30 represent the data from columns 5 and 7, respectively. The main objectives of the tests with these two columns were to study the effect of increased flow rate and reduced water volume on the release of ^{32}P from the cultivation chamber to the effluent chamber. Column 5 was maintained with a 50-ml volume of water above the sediment, whereas column 7 was operated with only a 25-ml volume of water above the sediment. Also, the flow rate of water to these columns was increased from 2.0 to 4.0 ml/hr; thus, the HRT in columns 5 and 7 was decreased from 24 hours to 12 hours and to 6 hours, respectively. It was then possible to study apparatus aberrations in the data due to flow rate, volume, and chamber wall effects. It was suspected that the ^{32}P released from the algal residue through bacterial degradation might have been precipitated or sorbed onto the walls of the cultivation chamber.

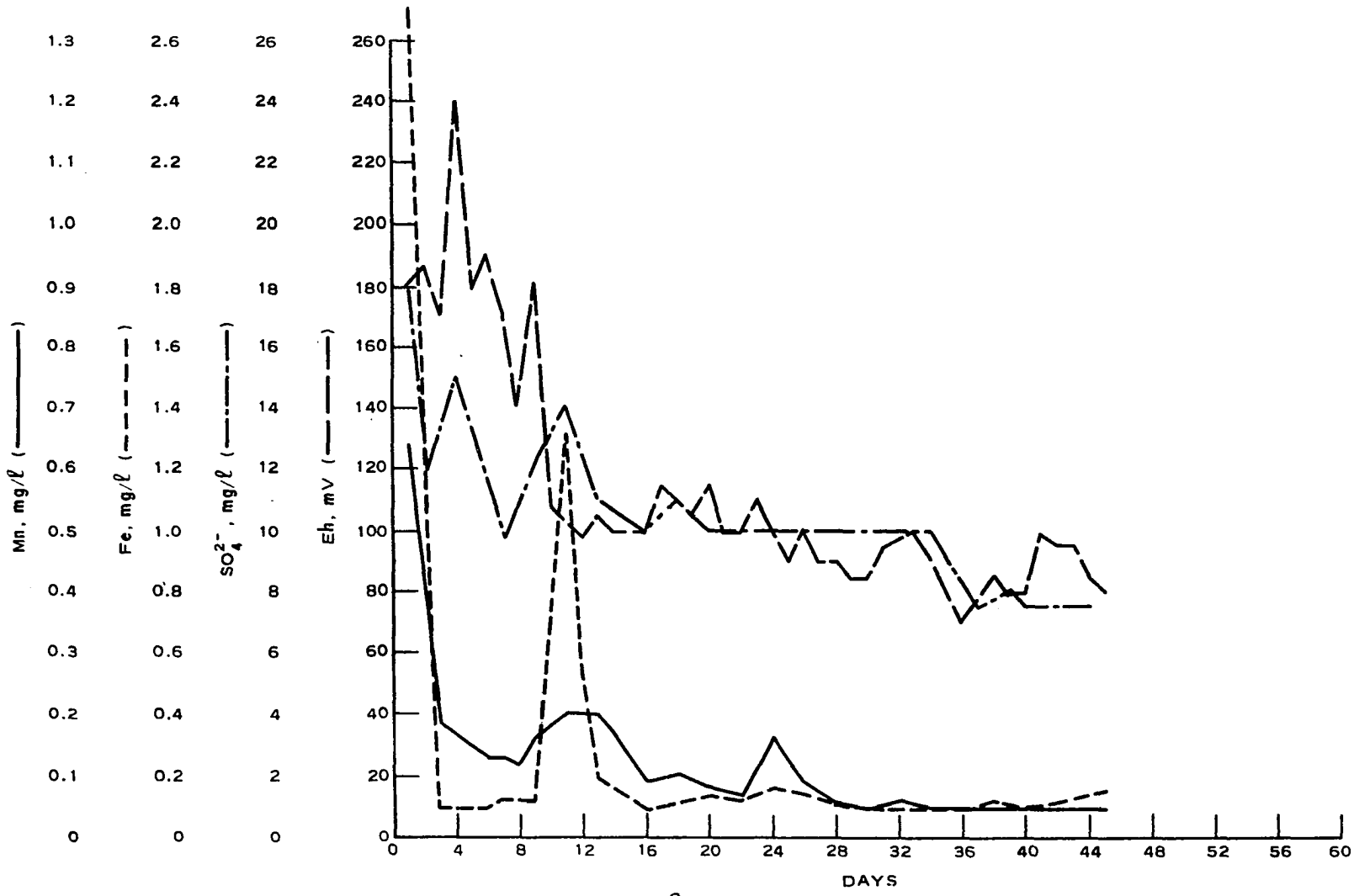


Figure 27. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 5 Effluent During Experimental Run 2

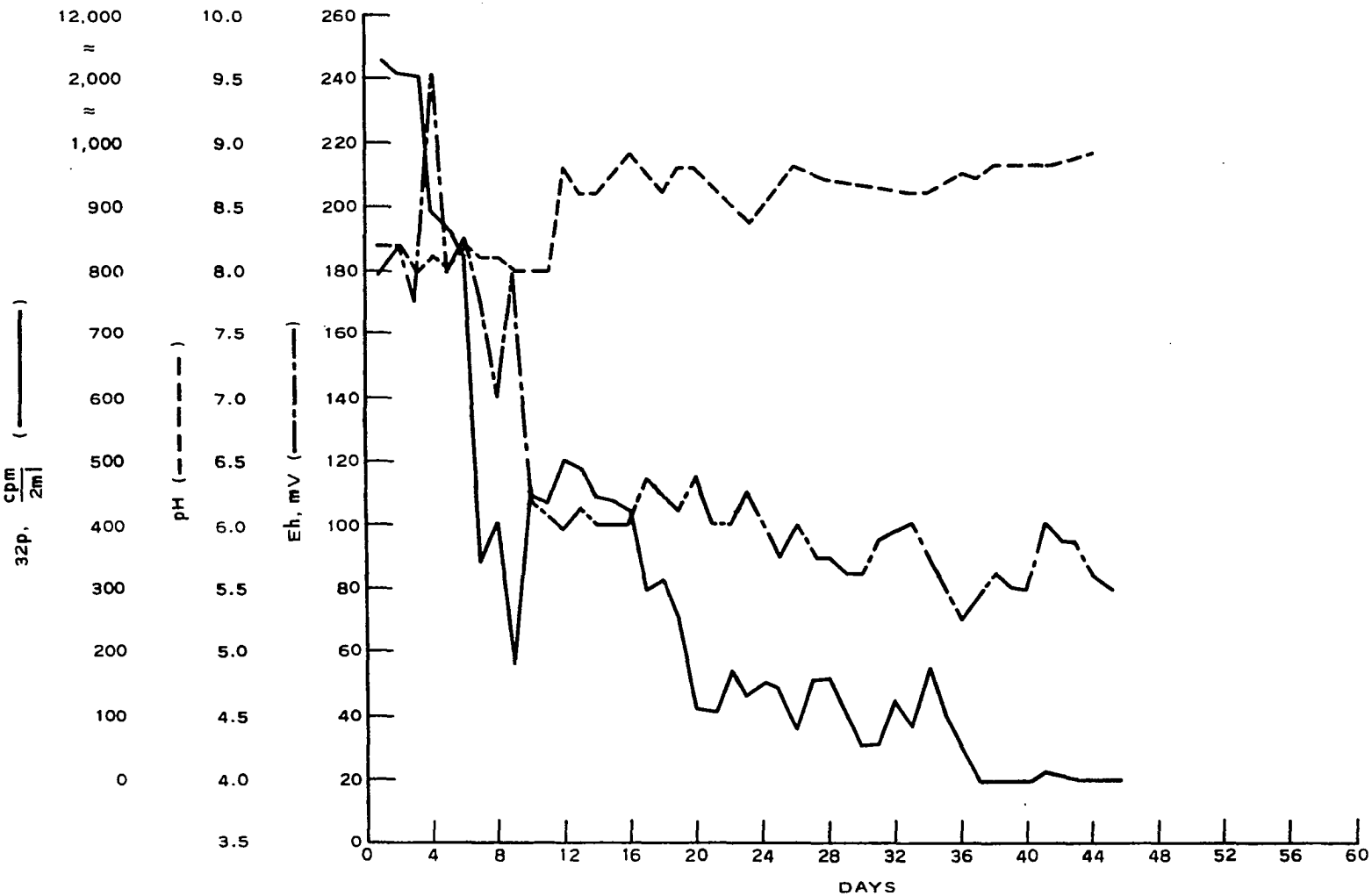


Figure 28. Comparison of ^{32}P, pH, and Eh for Column 5 Effluent During Experimental Run 2

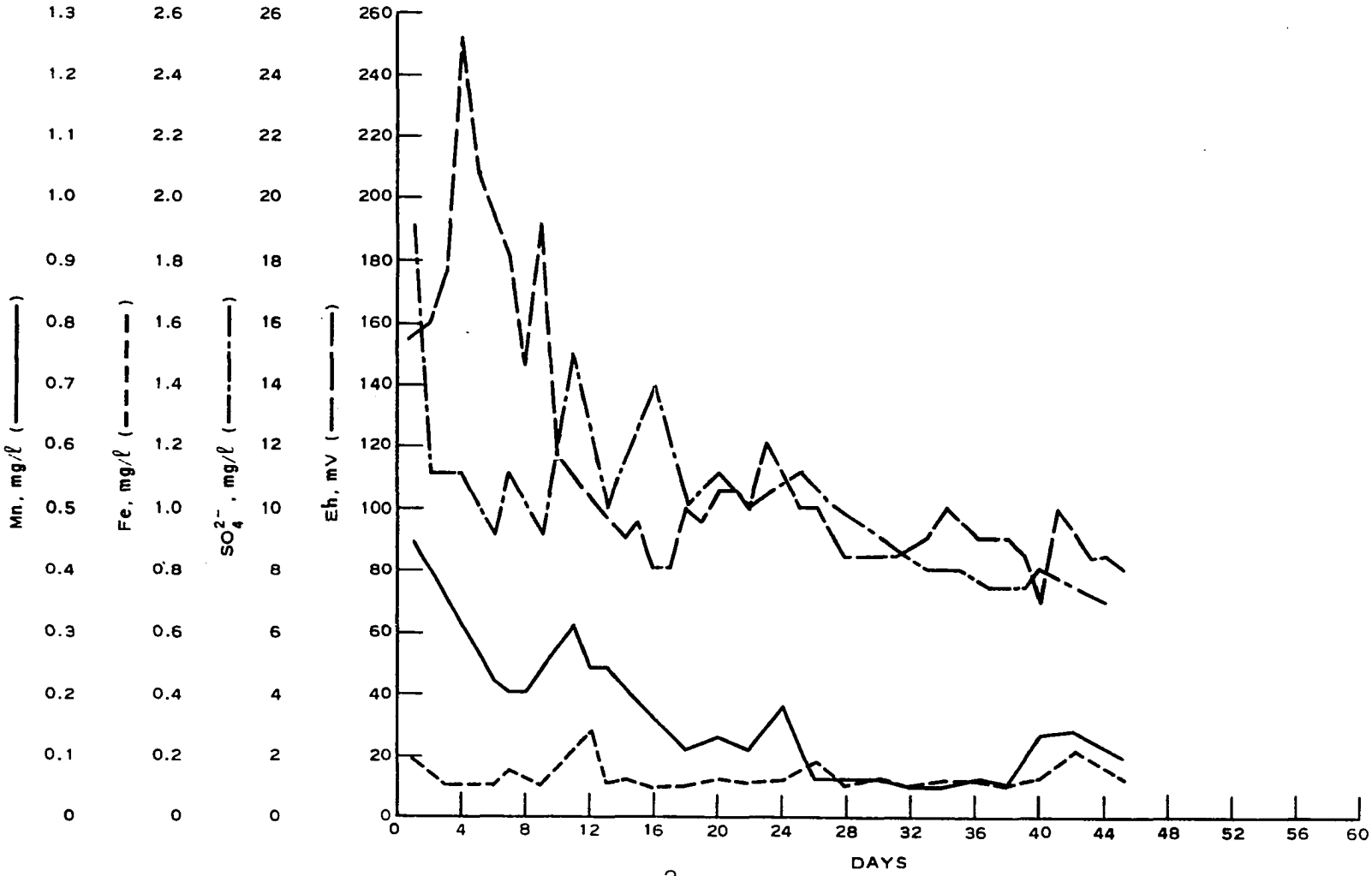


Figure 29. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 7 Effluent During Experimental Run 2

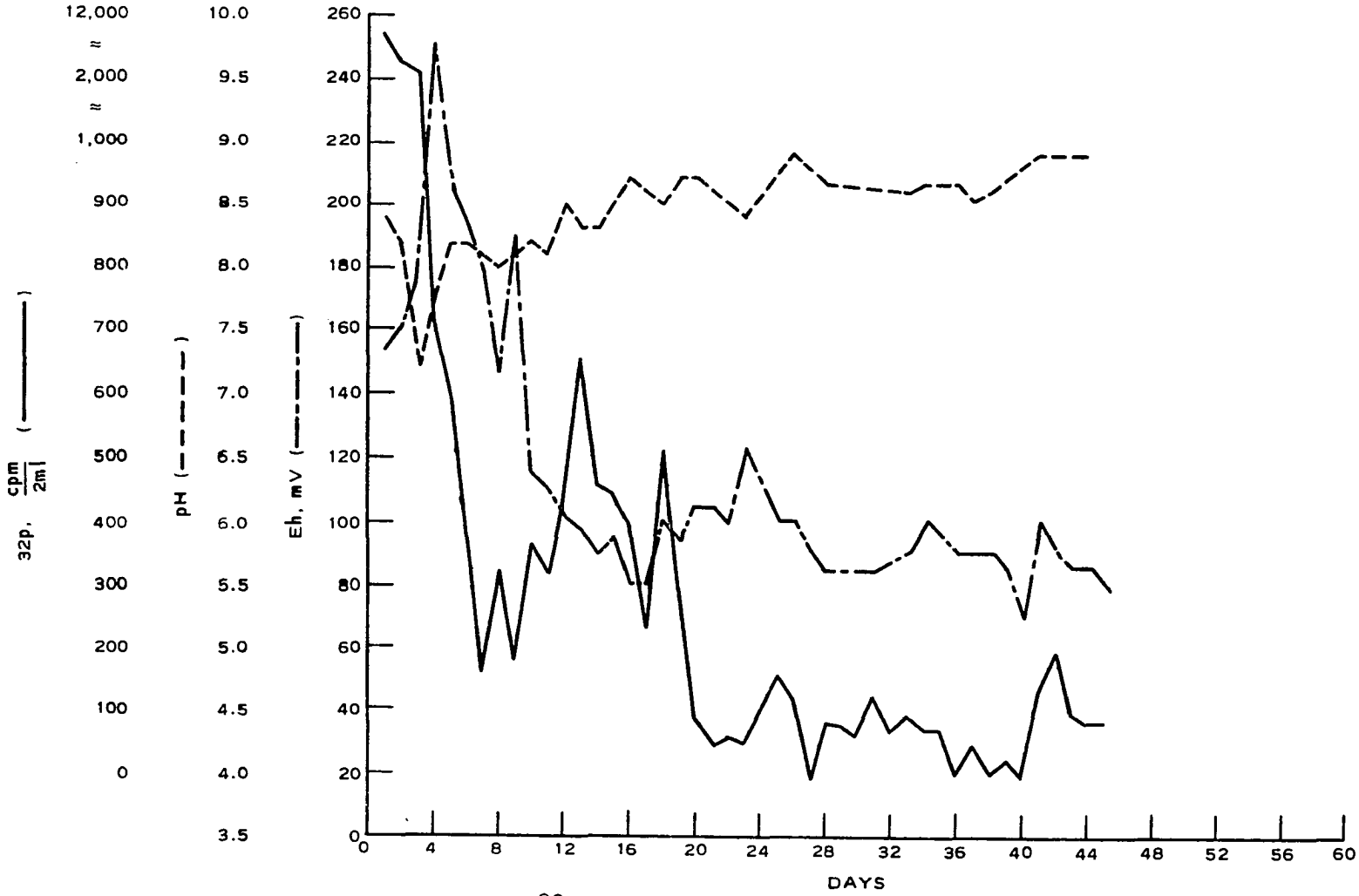


Figure 30. Comparison of ^{32}P, pH, and Eh for Column 7 Effluent During Experimental Run 2

The iron and manganese concentrations in the effluents from both columns, as shown in Figures 27 and 29, exhibited a similar pattern over the same time period. The effluent manganese and iron concentrations of column 5 rapidly decreased from days 1 through 3. The manganese concentration decreased from 0.65 mg/ℓ on day 1 to about 0.18 mg/ℓ on day 3, whereas the iron concentration decreased from 2.7 mg/ℓ on day 1 to less than 0.10 mg/ℓ on day 3. This washout was expected, as stated earlier, because of the possible disturbances created when the continuous-flow apparatus was being set up. The effluent manganese concentration from column 5 decreased slightly from day 3 to 0.12 mg/ℓ on day 8. Between days 8 and 16, the manganese concentration increased slightly to a maximum of 0.20 mg/ℓ on days 9 through 13; then, the effluent manganese concentration decreased to less than 0.10 mg/ℓ by day 16. From then on, it remained fairly constant through day 22. A slight manganese release from the cultivation chamber occurred between days 22 and 27. The maximum manganese concentration was measured on day 24 as 0.16 mg/ℓ. From day 28 through day 45, the effluent manganese concentration was less than 0.05 mg/ℓ.

A similar pattern in the effluent iron concentration from column 5 was observed; hence, the associated releases of iron and manganese were probably due to changes in the sediment redox potential in the cultivation chamber. From day 3 through day 9, the effluent iron concentration was less than 0.10 mg/ℓ. Between days 9 and 15, an iron release was observed. The maximum iron concentration in the column's effluent occurred on day 11 as 1.30 mg/ℓ. After day 11, a steadily decreasing effluent iron concentration was observed through day 16.

From day 16 through day 45, the iron concentration remained fairly constant between 0.10 and 0.18 mg/ℓ.

From comparing Figures 27 and 29, it was quite apparent that the effluent iron and manganese concentrations in column 7 were quite similar to those of column 5. The manganese concentration for column 7 decreased from 0.44 mg/ℓ on day 1 to 0.20 mg/ℓ by days 7 and 8. There was a slight increase to a maximum of 0.30 mg/ℓ on day 11. After day 11, a gradual decreasing manganese concentration was noted through day 18. From days 18 through 22, the manganese concentration was constant at about 0.12 mg/ℓ. There was a slight increase to 0.18 mg/ℓ on day 24 followed by a decline to about 0.05 mg/ℓ on day 26. Through day 38, the manganese concentration was constant. From day 38 to day 42, there was a slight increase in the manganese concentration. After day 42, the effluent manganese concentration decreased to 0.10 mg/ℓ by day 45.

The effluent iron concentration from column 7 did not vary significantly over the 45 days of operation. From day 9 through day 12, a slight release coinciding with the manganese release may have occurred. The iron concentration increased from about 0.10 mg/ℓ on day 9 to 0.28 mg/ℓ on day 12. Otherwise, the effluent iron concentration remained less than 0.20 mg/ℓ for the duration of the experimental run.

It was apparent that fairly anaerobic conditions existed in the cultivation chambers of both columns. Both the effluent sulfate concentration and the electrode potential decreased significantly over the time period of the experiment. From Figure 27, it can be seen that the effluent sulfate concentration from column 5 decreased from

18 to 12 mg/ℓ by day 2. From day 3 through day 13, the sulfate concentration was inconsistent. A possible explanation for this is that the redox potential at the sediment-water interface was unstable. After the observed release of iron and manganese from the column, the sulfate concentration remained constant at about 10 to 11 mg/ℓ from day 13 through day 34. After day 34, the sulfate concentration decreased further to less than 8 mg/ℓ. This latter decrease was probably due to a further decrease in the redox potential which resulted in an increased precipitation of hydrogen sulfide and/or ferrous sulfide.

A similar decrease in the effluent sulfate concentration of column 7 was observed. There was a rapid decrease from a sulfate concentration of 19 mg/ℓ on day 1 to 11 mg/ℓ on day 2. From day 2 through day 10, the sulfate concentration oscillated between 9 and 11 mg/ℓ. From day 11 through day 17, the sulfate concentration varied between 10 and 15 mg/ℓ. However, after the observed iron and manganese release from day 8 to day 18, the sulfate concentration stabilized at around 10 mg/ℓ from days 18 to 27. Following day 27, the sulfate concentration further decreased to about 8 mg/ℓ on day 33. After day 33, the sulfate concentration remained constant through the completion of this experimental run.

The effluent electrode potentials of columns 5 and 7 were also very similar. From day 1 through day 10, the electrode potential of column 5 oscillated greatly, but it generally decreased to about 110 mV by day 10. From days 10 through 24, the electrode potential remained fairly constant. There was a slight decrease to 80 mV on

day 35. From day 35 to day 45, the electrode potential averaged about 90 mV.

Likewise, a similar pattern was observed in column 7. From day 1 through day 12, the electrode potential oscillated greatly, but it still decreased to about 100 mV by day 12. From day 12 through day 45, the electrode potential remained fairly constant between 80 and 100 mV.

Figures 28 and 30 also indicate similar ^{32}P releases from both columns. In column 5, a rapid washout of ^{32}P is shown occurring as expected from day 1 through day 9, i.e., 4000 to 200 cpm/2 ml. From days 9 to 19, an increased ^{32}P release occurred. The maximum release was noted on day 12 when the effluent ^{32}P counting rate was 500 cpm/2 ml. After day 16, the activity of ^{32}P in the effluent decreased to 100 to 150 cpm/2 ml by day 20. The ^{32}P counting rate remained constant from day 20 through day 35. After day 35, only background levels of ^{32}P were counted in the effluent.

A similar washout of ^{32}P in column 7 occurred between days 1 and 7. Between days 7 and 9, the effluent ^{32}P activity oscillated between 180 and 320 cpm/2 ml. An increase in ^{32}P activity followed day 9 and reached a maximum of 650 cpm/2 ml on day 13. By day 20, the activity had decreased to less than 100 cpm/2 ml. Between days 20 and 45, the ^{32}P activity was fairly constant. A slight release might have occurred between days 40 and 42. The ^{32}P activity increased from background levels to about 200 cpm/2 ml at this time. By day 43, the ^{32}P activity was less than 100 cpm/2 ml.

The pH of the effluent from both columns gradually increased

from about 8.0 to about 8.7. Column 5 stabilized after day 16 at a pH of about 8.7. Column 7 attained a pH of 8.7 by day 12. The pH remained constant in both columns through the completion of the 45th day of the experimental run.

The major physical changes which occurred in these two columns were the same as those which occurred in the previous columns. The initial green algal residues in both of the columns were either coated with a black precipitate or they turned black within the first 2 weeks of this experiment. The black coloration appeared around day 8 or 9 in both columns.

In conclusion, the increased flow rate to both columns and the decreased water volume, along with a subsequent decreased HRT in column 5 and 7, did not alter the ^{32}P release from the columns. Furthermore, no effect was discernable on the patterns of release of the water quality parameters measured.

Columns 6 and 8

The data from columns 6 and 8 are shown in Figures 31, 32, and 33, 34, respectively. These two columns were operated under the same conditions as columns 5 and 7. The one exception was that an additional 0.5 mg P/ℓ as KH_2PO_4 was added to the lake water being passed through each column. The objective of tests with these two columns was to study the effect of increased ambient phosphorus in lake water on the accessibility of sediment phosphorus to the overlying water. In addition, the decreased HRT's and decreased water volume within the cultivation chamber of column 8 were necessary to evaluate the

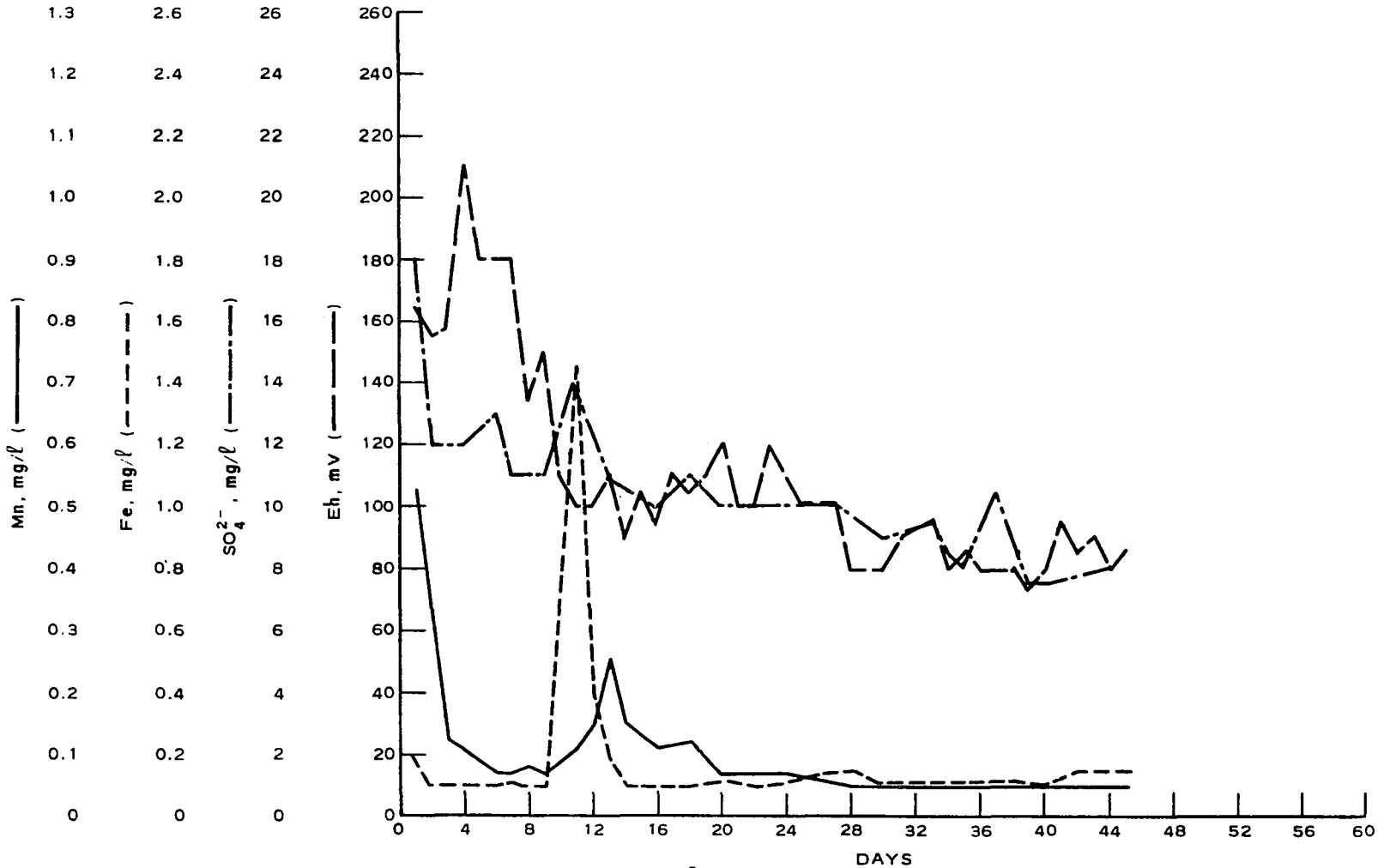


Figure 31. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 6 Effluent During Experimental Run 2

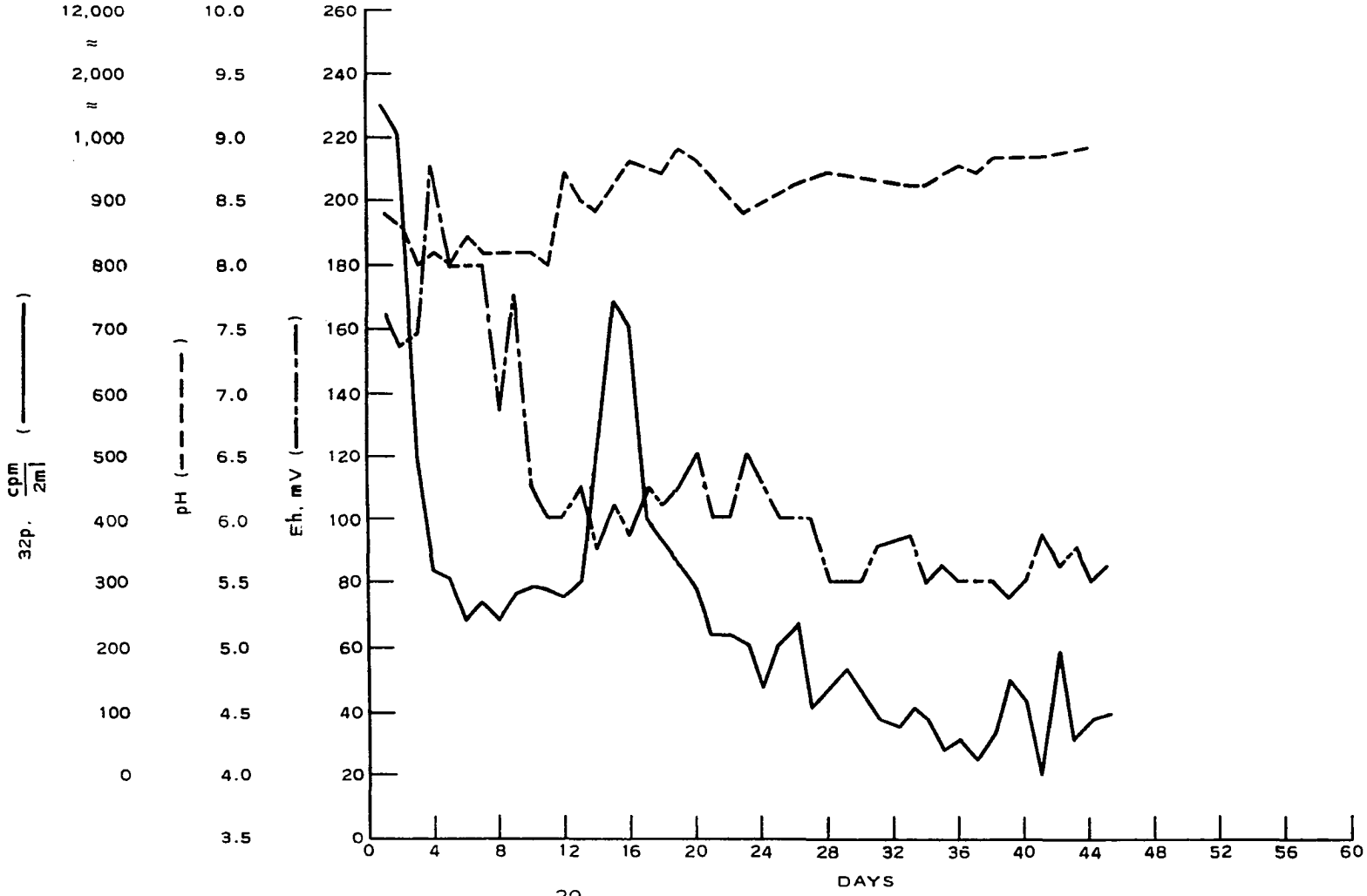


Figure 32. Comparison of ^{32}P, pH, and Eh for Column 6 Effluent During Experimental Run 2

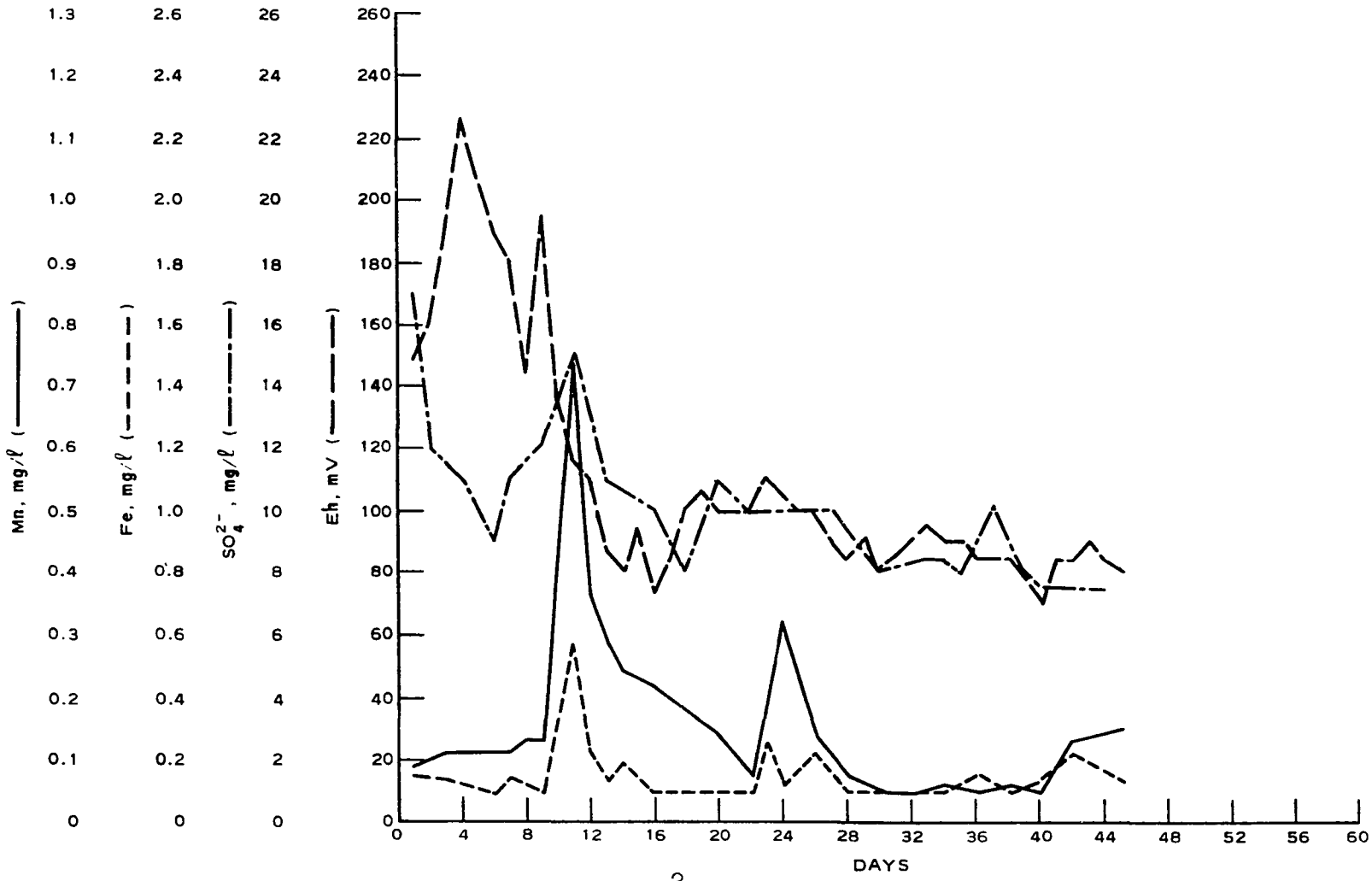


Figure 33. Comparison of Mn, Fe, SO_4^{2-}, and Eh for Column 8 Effluent During Experimental Run 2

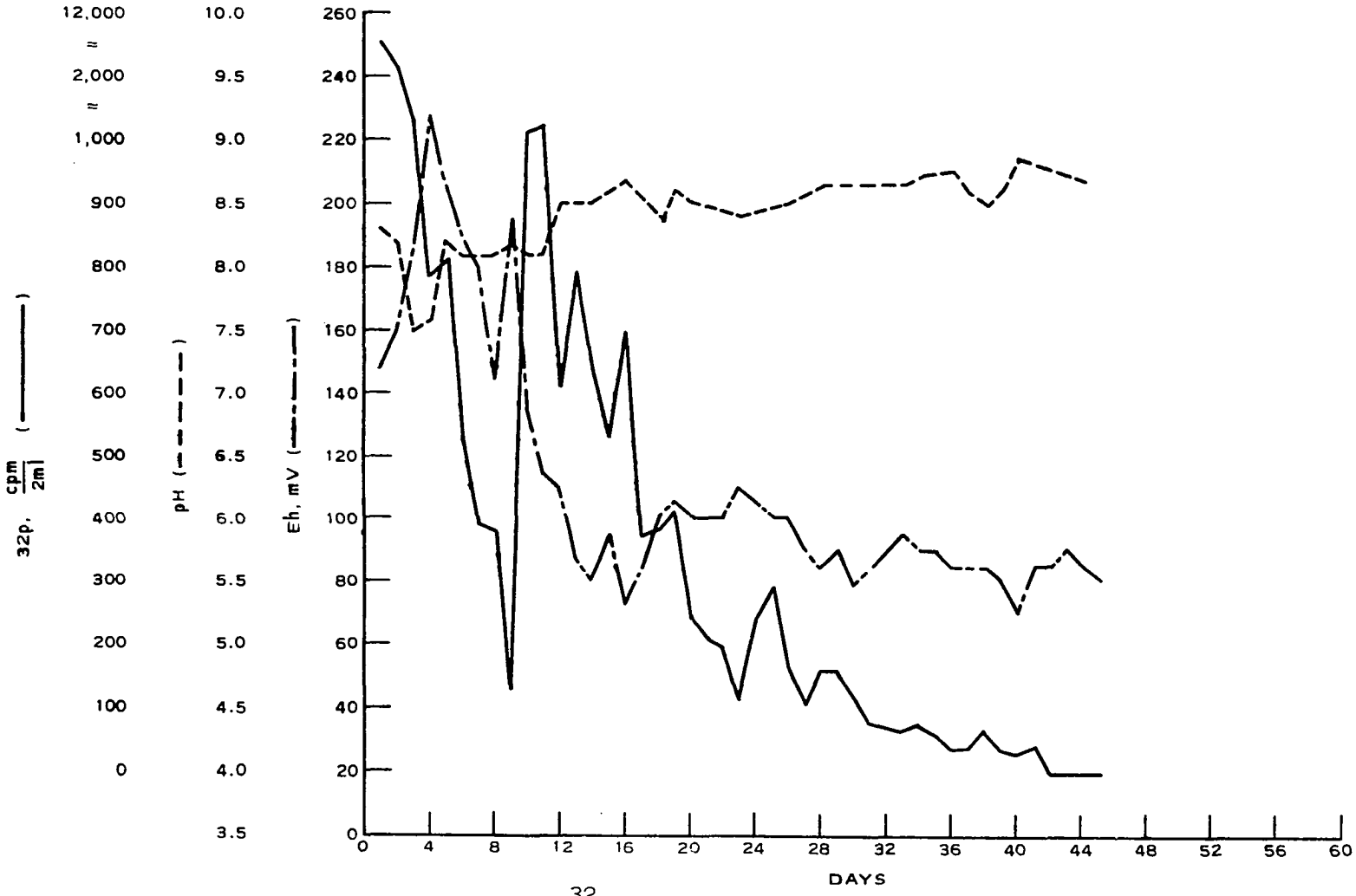


Figure 34. Comparison of ^{32}P, pH, and Eh for Column 8 Effluent During Experimental Run 2

apparatus effects on the behavior of the columns with respect to the stated water quality parameters.

From Figures 31 and 33, it can be seen that the effluent iron and manganese concentrations from both columns were very similar over the duration of the 45-day run. The effluent manganese concentration of column 6 decreased from 0.53 mg/ℓ on day 1 to 0.13 mg/ℓ on day 3. A further decrease through day 6 to 0.08 mg/ℓ was maintained through day 9. Between days 9 and 20, an increased release of manganese from the column was evident. The maximum manganese concentration of 0.26 mg/ℓ occurred on day 13. By day 20, the manganese concentration was down to 0.08 mg/ℓ. From day 20 through day 45, a manganese concentration of about 0.05 mg/ℓ was maintained.

A similar manganese release pattern was observed in column 8 with three exceptions. First, a much greater concentration of manganese was measured in the effluent of column 8 than in that of column 6. Second, there seemed to be a slight manganese release between days 22 and 28 and after day 40. Finally, there was no washout of manganese from days 1 through 3 as observed in column 6. This was probably the result of there being more clay attached to the sides of the column as the sediment was extruded to 10 cm in length. From day 1 through day 9, the manganese concentration remained constant at about 0.10 mg/ℓ. From day 10 through day 22, a significant increase in manganese content in the effluent of column 8 was noted. The maximum manganese concentration occurred on day 11. At this time, it was 0.73 mg/ℓ. By day 22, the manganese concentration was down to 0.08 mg/ℓ. Between days 22 and 27, the manganese concentration

increased to 0.32 mg/ℓ by day 24, and then it decreased back to 0.08 mg/ℓ by day 28. Until day 40, the effluent manganese concentration was stable at 0.05 mg/ℓ. From days 40 through 45, a slight release occurred. On day 45, the manganese concentration was 0.15 mg/ℓ.

The responses of effluent iron concentrations from both columns were very similar to the manganese releases. From days 1 through 9, the effluent iron concentration of column 6 was less than 0.10 mg/ℓ. A rapid increase in iron concentration was noted on day 11. At this time, the iron concentration was 1.40 mg/ℓ. On day 12, the iron concentration had dropped to 0.40 mg/ℓ in the column effluent. This decrease continued through day 14. From day 14 to the conclusion of the 45th day of operation, the effluent iron concentration remained less than 0.14 mg/ℓ.

In column 8, the increased iron concentrations in the effluent from the cultivation chamber occurred at about the same period of time. From day 1 through day 9, the iron content was about 0.10 mg/ℓ. After day 9, the iron concentration increased to 0.58 mg/ℓ by day 11, and decreased to 0.10 mg/ℓ by day 16. From day 16 through day 22, the effluent iron concentration was constant and very similar to that of the influent lake water (Appendix D). After day 22, a very slight iron release was observed. This apparent release lasted a few days with a maximum iron concentration of 0.24 mg/ℓ occurring on day 23. Minor releases were again noted on days 38 to 45 with a maximum iron concentration of 0.20 mg/ℓ on day 42. By day 45, the iron concentration had decreased to 0.12 mg/ℓ.

The decreasing sulfate concentration and electrode potential of the effluent from both columns over the duration of this experiment

strongly indicate that anaerobic conditions existed in both columns. In column 6, the sulfate concentration decreased from 18 mg/ℓ on day 1 to 12 mg/ℓ on day 2. From day 2 through day 9, the sulfate content was fairly constant. The effluent sulfate concentration increased to 14 mg/ℓ by day 11, and then decreased to 10 mg/ℓ by day 16. There was very little change through day 27. From day 28 through day 35, the sulfate concentration decreased slightly to about 8 mg/ℓ. On day 37, it had increased to about 10 mg/ℓ, and next it decreased to 8.0 mg/ℓ by day 39. No further changes occurred.

In column 8, the sulfate concentration decreased from 17 mg/ℓ on day 1 to about 9 mg/ℓ by day 6. After day 6, the effluent sulfate concentration increased to 15 mg/ℓ on day 11; then, it gradually decreased to 8 mg/ℓ by day 18. From day 18 through day 45, the sulfate concentration remained constant between 8 and 10 mg/ℓ.

The electrode potentials of the effluents from both columns oscillated between days 1 and 10, but they did decrease overall to about 100 mV by day 10. From day 10 through day 27, the electrode potentials of both columns remained constant at about 100 mV. After day 28, the electrode potential slightly decreased to about 80 mV in the effluent of both columns. No further electrode potential changes were significant through day 45 to warrant further comment.

Figures 32 and 34 show similar releases of ^{32}P from both columns. The initial washout of ^{32}P from column 6 occurred within the first 4 days. By day 4, the effluent ^{32}P activity was 300 cpm/2 ml, and remained fairly stable through day 13. From day 13 to day 20, there was a release of ^{32}P from the column. The maximum activity of

approximately 700 cpm/2 ml occurred on days 15 and 16. This was followed by a decrease in the rate of ^{32}P released from the column. The decrease was from 400 cpm/2 ml by day 17 to 300 cpm/2 ml by day 20. From day 20 through day 45, the effluent ^{32}P activity decreased to less than 100 cpm/2 ml.

From column 8, there was a rapid washout from day 1 through day 9 during which the ^{32}P activity in the effluent decreased from 7000 to 130 cpm/2 ml. On days 10 and 11, the effluent ^{32}P activity had increased to 1000 cpm/2 ml. Between days 11 and 23, the ^{32}P activity gradually decreased to about 130 cpm/2 ml by day 23. From day 23 through day 45, the effluent ^{32}P activity decreased further to background levels.

The effluent pH of columns 6 and 8 gradually increased to between 8.5 and 8.8 by day 12. From day 12 through day 45, the pH remained constant in both columns.

The algal residue in both columns began to turn black on day 11 or 12 and completely turned black by day 20.

In conclusion, the increased phosphorus concentration in the lake water in columns 6 and 8 seemed to have little effect on the rate or quantity of release of ^{32}P from the sediment via isotopic exchange reactions. The data from these columns were very similar to the data from columns 5 and 7. Also, a decreased HRT, resulting from increasing the flow rate of lake water to columns 6 and 8 and from decreasing the volume of water in column 8, did not significantly affect the water quality parameters measured in the effluents from these columns.

SUMMARY

Several column comparisons may be made from both experimental runs. These can only be qualitative comparisons, since very few replicate columns were handled in this experimental design. From most columns in both experimental runs, there was a definite physical washing out of some of the soluble organic ^{32}P, inorganic ^{32}P, and small amounts of particulate organic ^{32}P components of the algal cells. This was observed during the first several days of operation. Microscopic examination of 0.45-μ cellulose acetate filters, through which 1-ml aliquots of the column effluents were passed, did not indicate that algal cells were being washed out. However, some bacterial particulate ^{32}P associated with the suspended clays, may have been washed out of the columns, since some ^{32}P activity was on the filter. This washout was expected based on the aforementioned results of the boiling water extraction of the algal culture prior to the experimental runs. The extracted algal residue was not washed with distilled water prior to being placed in the columns, thus giving a similar washout of ^{32}P in most of the columns. The flow rate of the water and the HRT would typically regulate the rate at which this initial washout occurred. This was not noted in most of the column effluents. This washout of ^{32}P was considered to represent a very minor fraction of the total ^{32}P in the residue. There was approximately 1,000,000 dpm ^{32}P per extracted algal sample per column initially in the first experimental run, and approximately 6,000,000 dpm ^{32}P per extracted algal sample per column initially in the second experimental run. In fact, very

little ^{32}P was released from the sediment-water interface to the overlying water during both experimental runs (estimated to be <10 percent of the total ^{32}P activity).

General comparisons of the data from both experimental runs could be made concerning the effects of several environmental changes on the release of ^{32}P from the sediment and on the subsequent effluent water quality over a period of time. A comparison of the effluent water quality parameters (Fe, Mn, and SO_4^{2-}) from both natural and sterile columns indicated that Fe and Mn were substantially higher in the effluent of natural columns.

The sediment heterotrophic community was important in mediating the release of iron and manganese and in the reduction of sulfate to sulfide. Similarly, the release of ^{32}P from the viable columns of both experimental runs, through which lake water was passed, was associated with the release of iron and/or manganese. Since the release of the latter metals from the sediment is redox sensitive, the associated release of the ^{32}P indicates that a chemical complexing of these elements did occur probably between the range of oxidized to reduced conditions characteristic of these metals. It may also indicate that the redox condition in the water of each column fluctuated above and below that potential necessary for the reduction and solution of the chemical complex. The atmosphere in the cultivation chambers was initially air during experimental run 1, and a nitrogen atmosphere was used in the second experimental run. Consequently, the measured electrode potentials during the first run did not indicate the trend of the redox conditions except after day 38. Nitrogen was used to replace the air

atmosphere after day 38 in columns 1 and 6 of experimental run 1.

During experimental run 1, the iron, manganese, and ^{32}P releases of columns 1 and 2 usually occurred over the same time period. Columns 5 and 6 showed releases of iron and manganese similar to those of columns 1 and 2, but the ^{32}P release was fairly constant over the duration of the experiment. Since distilled water was passed through columns 5 and 6, it appears that the sediment assemblage of organisms may have been lacking sufficient nutrients to maintain active growth. Consequently, the extracellular enzymatic breakdown of particulate organic ^{32}P, which normally would enable the other organisms to utilize the available ^{32}P, would continue. The ^{32}P would be washed from the sediment without being utilized by the normally active growing community. The analysis of the effluent from column 7, which was sterile, indicated that without the viable organisms to "mineralize" the particulate organic ^{32}P then ^{32}P could not be released to the overlying water from the sediment. Furthermore, the microorganism's importance in mediating redox conditions was made obvious since no iron or manganese was released. Columns 3 and 4 indicated a similar response prior to day 30 or 31. After formalin was added, iron and manganese was released, but ^{32}P was still not released.

In the second experimental run, the volume and *HRT* were manipulated, as well as the ambient phosphorus concentration in the lake water that was passed through columns 2, 4, 6, and 8. As stated earlier, iron, manganese, and ^{32}P releases similar to those shown in the previous run between days 8 and 16 occurred in most of the columns. The specific activity of ^{32}P was increased in order to obtain better

statistical counting rates over the duration of the run. Consequently, the ^{32}P activity in the effluent was expected to be greater than it was in the previous run. The effluent ^{32}P in columns 2 and 4, which had the same volume and HRT as the previous run, remained much higher over the first few days of operation. This was not apparent in columns 6 and 8.

The aerobic versus anaerobic atmosphere in the cultivation chambers had practically no effect on the amount of ^{32}P that was detected in the effluent. After day 38 in column 1 of experimental run 1, there was a release of manganese along with some ^{32}P. However, this release was probably a result of reducing the Mn-P complex that was precipitated onto the walls of the polycarbonate tube or glass overflow tube. This was further indicated after day 38, when a slightly brown precipitate was visually noted on the overflow tube.

Upon purging the columns with nitrogen, there was also an associated increase of the pH in the column effluents because carbon dioxide diffusion into the water was prevented. Under an aerobic atmosphere, the water was apparently being reaerated (i.e., with oxygen and carbon dioxide) as it flowed out of the cultivation chamber and into the effluent chamber. This would account for the slightly lower pH values due to carbonate buffering. At the pH values recorded in the effluent from the columns, precipitation of $^{32}PO_4^{3-}$ as hydroxyapatite was also considered as a possible explanation as to why very little ^{32}P was released. However, at the decreased HRT's and the increased flow rates, the precipitated ^{32}P, as hydroxyapatite, should have been washed out of the columns. The formation of hydroxyapatite is a

metastable surface precipitation. Consequently, bacterial or clay particles being washed out of the column should have carried the ^{32}P out of the columns.

In all of the effluents from natural columns with lake water, the sulfate concentration generally decreased. However, no sulfate was detected in the effluents from those columns through which distilled water flowed. This was not entirely expected even though the sulfate concentration of distilled water was 0.0 mg/ℓ, since there are numerous microorganisms capable of oxidizing any available sulfides, elemental sulfur, thiosulfate, tetrathionate, and, in some cases, sulfite to sulfate. The heterotrophic microorganisms involved are a poorly defined group of bacteria, fungi, and actinomycetes.

Other parameters which were monitored regularly but which are not included in the general discussion were ammonia, nitrogen, and total dissolved solids. As stated earlier, the ammonia nitrogen determination was discontinued after about 2 weeks had elapsed in both experimental runs. The Nesslerization technique would only work in sterile columns and in viable columns through which distilled water was passed. The data showed negligible ammonia concentrations in those columns maintained with distilled water. The total dissolved solids were determined regularly on the effluent using a Myron Dissolved Solids meter. This meter is not sensitive enough to detect subtle changes in total dissolved solids of the effluent. These small changes in dissolved solids were a consequence of having such a small surface area of sediment exposed to the overlying water.

CHAPTER V

ATTEMPTS TO FRACTIONATE THE SEDIMENT ^{32}P

Sample Preparation

At the conclusion of the 45th day of the second experimental run, it was quite evident that the general trend of the column effluent analyses were similar to that of the previous experimental run. This run was then terminated. The columns were dismantled in order to attempt to fractionate the sediment inorganic ^{32}P and organic ^{32}P. This determination would indicate whether or not the particulate organic ^{32}P was degraded by the heterotrophic assemblage of organisms.

The columns were carefully removed in order to avoid disturbing the sediment-water interface. Next, each cultivation chamber was opened from the top of the column. Most of the water overlying the sediment was removed from each column using a syringe with a rubber tube attached to the end. After removing the water and the surficial sediment (0- to 1-cm depth), a few milliliters of distilled water were added to the tubes in order to aid in fluidizing the surface sediment; thus, making it easier to remove each successive 1-cm sediment aliquot. A core depth of 3 cm was removed and separated into sterile 125-ml Erlenmeyer flasks.

The layers represented approximately the 0- to 1-cm depth, the 1- to 2-cm depth, and the 2- to 3-cm depth. The 0- to 1-cm depth represented the bulk of the labelled algal bodies. This layer had a very deep black color. The coloration was obvious in all eight columns. Associated with this layer was a very strong sulfide odor indicating that the redox condition in the sediment was indeed much more reducing than was indicated by the electrode potential in the overlying water. The electrode potential of this black layer and the lower layers was measured for each column.

From the results shown in Table 7, the electrode potential in the 0- to 1-cm sample measured between -100 to -150 mV after a 15-minute equilibrating time. These electrode potentials, along with the pungent hydrogen sulfide odor, indicated that the sediment-water interface was anaerobic in all eight columns. Below the 1-cm depth, the sediment electrode potentials were not as low. Hence, most of the biologically mediated reduction processes occurred in the surficial 1 cm of sediment.

After determining the electrode potential for each column, the sediment samples representing the 2- to 3-cm depth were frozen. The other samples were refrigerated until needed.

Several methods were attempted to obtain the particle-size distribution of the sediment as well as the organic-inorganic component separations. At first, several methods were selected to conserve the particulate organic and inorganic constituents in their natural state. Since these methods did not yield satisfactory results, chemical extraction of the sediment according to Mehta et al. (1954)

was felt to be the best alternative. This procedure sacrificed the sample as far as attempting other separation techniques were concerned.

All ^{32}P counting rates reported in the following discussion were not corrected for decay or counter efficiency. The same internal proportional counter was used for all samples. Also, counting efficiency, with sediment plus ^{32}P and algae plus ^{32}P, was determined by noting decreasing sample count with increasing weight of sediment and algae. To a set of three tared planchets containing approximately the same ^{32}P activity, increasing increments of unlabelled algae or sediment were added. After each addition of algae or sediment, the planchets were dried, weighed, and counted. This was continued until selfabsorption effects were significant. Based on this, all sample counting (mentioned in the fractionation attempts) was performed on an aliquot in which selfabsorption was negligible. It was not necessary to correct for ^{32}P decay, since comparisons of fractionation methods were based on ability to separate the organic ^{32}P components and the inorganic ^{32}P components, and all compared samples were counted within a short time relative to the half-life of ^{32}P.

Filtration

Filtration of the sample, through a series of preweighed Millipore filters of different sizes ranging from 5 to 0.22 μ , was thought to be a good procedure for sizing the particles in the sediment sample. It would also allow microscopic examination of the filters to observe the microbial components. Finally, through reweighing of the filter plus sample and the ignition of the respective filter and

sample at 600°C for 1 hour in a muffle furnace, it would be possible to obtain an organic-inorganic ratio of the particulates by weight comparison. This would hopefully enable the association of the ^{32}P counts with either the organic or the inorganic particulates based on the ratio of ^{32}P to each component. This was not performed in this case because of the problems associated with the filtering process.

Six different filtering series were utilized in the following manner on column 1 at the 0- to 1-cm depth:

Series No.

1-----A 2-ml aliquot from the completely mixed sample was filtered through two 5-μ Millipore cellulose acetate filters successively.

2-----A 1-ml aliquot was filtered through 5-, 1.2-, 0.8-, 0.45-, and 0.22-μ cellulose acetate filters.

3-----Approximately 1 ml was filtered through two 1.2-μ cellulose acetate filters successively.

4-----One milliliter of the sample was filtered through 0.8-0.45-, and 0.22-μ filters in succession.

5-----One milliliter of the sample was filtered through 0.45- and 0.22-μ filters.

6-----One milliliter of the sample was filtered through a 0.8-μ filter.

The results from these filtering attempts are shown in Table 8.

There were no detectable particulates being filtered beyond the first filter. All filters were pretreated with a stock phosphorus

TABLE 8

RESULTS OF FILTRATION ATTEMPTS ON COLUMN 1, SEDIMENT REPRESENTING THE 0- TO 1-CM DEPTH

Series No.	Pore size				
	5 μ	1.2 μ	0.8 μ	0.45 μ	0.22 μ
1	366 cpm* BKG.				
2	872 cpm	40 cpm	32 cpm	30 cpm	13 cpm
3		379 cpm BKG.			
4			924 cpm	40 cpm	38 cpm
5				212 cpm	44 cpm
6			488 cpm		

* All ^{32}P counts in this table were averages of three 10-minute counts.

solution to minimize sorption of ^{32}P onto the filter.

The remainder of the 0- to 1-cm layer was filtered through a No. 325 standard 3-inch sieve (45-μ pore size). The residue was dried in an oven on a planchet and then counted for ^{32}P using the same internal proportional counter operating at 1950 V. The ^{32}P counted was the average of three counts of 10 minutes each. An average count of 68 cpm was recorded for the residue.

It would seem that the majority of the ^{32}P was associated with the particulates ranging from less than 45 to 5 μ. However, the filtration series resulted in a clear filtrate after passing through any one of the filters. This could be deceiving because the fine colloidal-size clays tended to agglomerate on the larger pore-size filter and did not pass through. Some finer particles (0.45 μ) were

known to be there because centrifugation at 10,000 rpm for 10 minutes in 35-ml volume did not settle all of these fine particulates. The sulfide present in the sediment may have caused the particles to agglomerate; therefore, filtration was discarded as a means of separation.

Centrifugation

The sediment from column 2, consisting of the black layer (0 to 1 cm) and the clay layer (1 to 2 cm), was centrifuged on a Sorvall centrifuge at various combinations of speed and time.

It was found that the majority of the clumps of sediment settled out at less than 500 rpm in 5 minutes for a volume of 35 ml. But mixing and resuspending in fresh, distilled water resulted in more of the sediment remaining in suspension after the same time and rpm setting.

Since clear separation was not attainable, it was thought that some dispersing agent might be used to break the clumped particles into discrete particles and thus allow for centrifugation or filtration to separate the particles.

Dispersing Agents

Since differential centrifugation did not adequately separate particle sizes, several pretreatment procedures were followed. Urea (6 to 8 M) is typically used in microbiology to disperse enzyme material by reducing the hydrogen bonding characteristic of organic-inorganic complexes. Calgon (sodium hexametaphosphate) was also used, since it is commonly used to disperse soil particles prior to particle

sizing. Sonication was the last method to be used because it is destructive to the microorganisms in the sediment-water mixture. The procedures for sample 2 were as follows:

Urea

(1) Enough urea was added to the sample flask to represent an 8-M solution.

(2) The flask was shaken for 12 hours on a wrist-action shaker.

(3) A 2-ml aliquot was then passed through an 8-μ filter. The filter and residue were then counted three times for 10 minutes.

(4) Next, the filtrate was passed through a 0.45-μ filter, evaporated, and counted as above.

(5) The bulk of the solution was then centrifuged.

The ^{32}P activity retained on the 8-μ filter was 567 cpm. However, only background activity was counted on the 0.45-μ filter. The sediment was not well dispersed, since centrifugation at less than 500 rpm for 5 minutes settled most of the material out of solution. Upon resuspending the sediment with distilled water and centrifuging under the same conditions, increased turbidity remained in the aqueous phase. This indicated that the sediment particles were still agglomerated and were not separating discretely.

Calgon

Calgon was used next because of its wide acceptance as a dispersing agent for soil particle-size analysis. The following procedure was followed on column 2 at 0- to 1-cm and 1- to 2-cm subsamples.

(1) Twenty-five ml of calgon solution were added to the sample flask.

(2) Next, the flask was shaken for 18 hours.

(3) Differential centrifugation was again attempted.

The bulk of the sediment settled out after 5 minutes at 500 rpm. Reducing the time of centrifugation did not seem to aid the settling characteristics. Each time the sediment was resuspended in distilled water and recentrifuged at 500 rpm, the supernate was very cloudy. This was repeated four times with the same problem encountered each time.

Consequently, separation of the different particle sizes was not attainable via centrifugation following the sediment dispersion in calgon.

Sonication

Since the latter two dispersing techniques did not seem to work, sonication on the same samples was then attempted. By varying the times for which the sample was exposed, it was thought that the organic and inorganic components would be separated. This belief was based on their differential densities and physical structures. The following procedure was used:

(1) The sample was transferred to a stainless steel beaker and placed in the sonicating unit.

(2) Exposure was varied: 5 minutes, 10 minutes, 20 minutes, 30 minutes, 45 minutes, and 60 minutes.

(3) After each time period, differential centrifugation was

attempted in order to separate the particle sizes and thus to minimize any agglomeration of the particles.

No clear separation of particle size was attained by means of the centrifuge after all of the time periods. To break up the clay particles into their basic physical units, exposure would have to have been extended well past 1 hour. This technique did not seem appropriate since cellular destruction would have been essentially complete even at the longer exposure times already used.

Weight Comparison

The basis for this technique for locating the ^{32}P as part of the organic or inorganic material in the sediment was to obtain a ratio of organic to inorganic material. The quantity of inorganic material was obtained by igniting an aliquot of sediment for 1 hour at 600°C. The quantity of organic material was determined by the weight loss of the sample measured before and after the ignition process, the idea being that the organic components would be volatilized at 600°C.

This ratio coupled with ^{32}P counts would give some idea at least as to whether the ^{32}P was organic or possibly associated with the inorganic fraction. The procedure was as follows:

(1) The black layer (0 to 1 cm) and the clay layer (1 to 2 cm) of column 5 were mixed with a magnetic stirrer in a flask with a total volume of approximately 50 ml.

(2) Several aliquots of different sizes were taken from each flask and placed on preweighed Millipore filters in planchets.

(3) The aliquots were dried in a planchet oven and counted using the proportional counter.

(4) The filters and samples were then weighed.

(5) Preweighed crucibles were then used to hold the filters in the muffle furnace.

(6) The crucible containing the filter and sample was weighed prior to ignition and after ignition.

This weight comparison seemed to be inconclusive (Table 9).

TABLE 9

RESULTS OF THE SEDIMENT WEIGHT COMPARISON ANALYSIS FOR COLUMN 5

Inorganic sample weight, mg	Organic sample weight, mg	Aliquot	Ratio inorganic: organic	Net cpm*	Inorganic cpm/mg	Organic cpm/mg
		0- to 1-cm Depth				
3.6	0.4	1 ml	100:11	158	43.9	395.0
10.2	1.0	2 ml	100:10	450	44.1	450.0
13.5	0.6	4 ml	100:4	483	35.8	805.0
21.9	2.7	6 ml	100:12	705	32.2	261.1
22.1	2.1	8 ml	100:10	655	29.6	311.9
40.7	6.2	12 ml	100:15	1176	28.9	189.7
		1- to 2-cm Depth				
29.9	2.1	1 ml	100:7	95	3.2	45.2
102.0	9.1	2 ml	100:9	306	3.0	33.6
129.4	10.5	4 ml	100:8	416	3.2	39.6
100.0	105.7	6 ml	100:108	528	--	--
101.6	122.3	8 ml	100:120	647	--	--
32.7	57.1	10+ ml**	100:175	343	--	--

* Net count represents average of three 10-minute counts.
** More water than sediment.

Based on the counts per minute per milligram of organic material and inorganic material, it would seem that the ^{32}P was generally

associated with the inorganic components. This conclusion is mainly the result of analyzing the spread of the counts per minute per milligram values. The larger the spread in the ratios, the less likely that the ^{32}P would be associated with that fraction. Theoretically, a uniform quantity of ^{32}P would be associated with a unit weight of similar material. Thus, increasing the weight of the material on the filter would give a constant ratio of ^{32}P per unit weight. For the inorganic material in the black layer, the range was 15 cpm/mg inorganic (factor <2) between the lowest and highest values. For the organic material, the range was greater than 600 cpm/mg organic (factor of 4) between the lowest and highest values.

The same general conclusion can be applied to the results on the clay layer. For the inorganic material, the spread was 0 to 2 cpm/mg inorganic (factor of <0.1), whereas, for the organic material, the range was 12 cpm/mg inorganic (factor of <0.3) between the lowest and highest values. Larger aliquots were removed from the clay layer and analyzed. However, the 6-, 8-, and 10-ml aliquots from the clay layer disturbed the ratio of inorganic to organic material. These three aliquots were removed from a small volume of sample remaining in the flask. Any clumps of organic matter which were not broken up by mixing, etc., would have a higher chance of being pulled out of the flask. An error may also have been made in recording the weights of the filters from these three samples. As a result, errors in organic material weights probably occurred. These last three samples were disregarded.

Again, these results were inconclusive with respect to

distinguishing organic from inorganic ^{32}P. They did indicate that the ^{32}P was confined primarily to the top 2 cm of sediment. A statistical analysis with replicate samples would be one way in which this type of data could be analyzed. Future work should consider this aspect.

The sediment samples representing the 2- to 3-cm depth from the eight columns were removed from the freezer and thawed. The same procedure as that described above was followed, except that only aliquots of 2 and 4 ml were taken from the samples. The results are shown in Table 10.

TABLE 10

RESULTS OF THE SEDIMENT WEIGHT COMPARISON ANALYSIS FROM THE 2- TO 3-CM SEDIMENT DEPTH OF EACH COLUMN

Sample	Aliquot	Inorganic sample weight mg	Organic sample weight mg	Ratio Inorganic: organic	Net cpm*	Inorganic cpm/mg	Organic cpm/mg
1	2 ml	93.8	16.8	100:18	BKG		
	4 ml	196.0	18.9	100:10	264	1.3	13.9
2	2 ml	118.9	12.7	100:11	65	0.6	5.0
	4 ml	154.2	21.2	100:14	109	0.7	5.2
3	2 ml	53.5	5.9	100:11	29	0.6	4.8
	4 ml	125.1	12.2	100.10	135	1.1	11.3
4	2 ml	263.5	45.2	100.17	25	0.1	0.6
	4 ml	328.3	38.0	100.12	85	0.3	2.2
5	2 ml	109.1	26.7	100.24	119	1.1	4.4
	4 ml	197.9	16.5	100.8	130	0.7	7.9
6	2 ml	142.8	15.9	100.11	224	1.6	14.0
	4 ml	127.2	17.5	100.14	164	1.3	9.1

(Continued)

* Average of three 10-minute counts.

TABLE 10 (Concluded)

Sample	Aliquot	Inorganic sample weight mg	Organic sample weight mg	Ratio Inorganic: organic	Net cpm	Inorganic cpm/mg	Organic cpm/mg
7	2 ml	153.3	18.2	100.12	139	0.9	7.6
	4 ml	290.4	36.5	100.13	198	0.7	5.4
8	2 ml	167.3	19.1	100.11	95	0.6	5.0
	4 ml	212.4	24.7	100.12	94	0.4	3.8

Sediment Phosphorus Extraction According to Mehta

Mehta et al. (1954) developed a procedure for determining organic phosphorus in soils. The procedure has been generally applied to sediments by many investigators since that time. It is now a well established technique for sediment organic phosphorus determination. Sommers et al. (1970) compared the Mehta procedure with several other procedures that have been developed recently for sediment-phosphorus fractionation. They showed that this procedure was most applicable to the determination of total organic phosphorus in sediments.

The procedure consists of a series of successive extractions with concentrated HCl and 0.5-N NaOH at room temperature, and 0.5-N NaOH at 90°C. The difference in the amount of inorganic phosphorus and total phosphorus in the combined extracts is considered to be the total organic phosphorus. This procedure was followed for analyzing columns 4, 6, and 8.

(1) The black layer (0 to 1 cm) and the clay layer (1 to 2 cm) of each sample were centrifuged at 1250 rpm for 10 minutes, and the supernate was decanted.

(2) Then 15 ml of concentrated HCl were added to each tube containing the sediment (approximately 1 g).

(3) These tubes were heated on a steam plate for 10 minutes. Final solution temperature was 70°C.

(4) Next, 15 ml of concentrated HCl were added and allowed to stand at room temperature for 1 hour.

(5) Fifty milliliters of distilled water were then added and mixed.

(6) The solution was centrifuged at 1250 rpm for 10 minutes, and the supernate was poured into a 250-ml volumetric flask containing 50 ml of distilled water.

(7) Thirty milliliters of 0.5-N NaOH were then added to the tube, stirred, and allowed to stand at room temperature for 1 hour.

(8) The tube containing the NaOH and the residue was centrifuged at 1250 rpm for 10 minutes. Then the supernatant was poured into the volumetric flask with the acid extract.

(9) Sixty milliliters of 0.5-N NaOH were added to the tube with the residue and stirred, and the tube was covered with an inverted 50-ml beaker.

(10) Each tube was heated in an oven at 90°C for 8 hours. The tubes were then cooled and centrifuged at 1250 rpm for 10 minutes, and the supernate was added to the volumetric flask with the previous extracts.

(11) The volumetric flask now contained both the precipitated

organic matter (organic ^{32}P) and the inorganic ^{32}P in solution.

(12) The flask contained a total volume of 220 ml. The contents were agitated and then divided into two 110-ml volumes.

Each of the samples was analyzed according to this basic procedure with a slightly different preparation for counting. The supernate after centrifugation was considered to contain mainly inorganic ^{32}P. The combined extract subsamples contained both organic ^{32}P and inorganic ^{32}P; hence, the counting rate was a measure of total ^{32}P from these particular samples.

Column 4

(13) One of the subsamples (110 ml) was centrifuged at 1250 rpm for 15 minutes.

(14) The supernate was poured into a 250-ml beaker and evaporated to a 20-ml volume (inorganic ^{32}P).

(15) The sample was then neutralized with 5 ml concentrated NaOH solution.

(16) The other 110-ml subsample was evaporated to 20 ml and neutralized with 5 ml concentrated NaOH (total ^{32}P).

(17) Three 1-ml aliquots were then evaporated to dryness in individual planchets using a heat lamp.

(18) The planchets from each sample were then counted in the internal proportional counter at 1950 V three times for 10 minutes each and the counts were averaged.

Column 6

(13) A 25-ml aliquot was removed from each of the two 110-ml well-mixed subsamples.

(14) One 25-ml aliquot was neutralized with concentrated NaOH and evaporated to 10 ml (total ^{32}P). (a)

(15) The other 25-ml aliquot was centrifuged at 1250 rpm for 10 minutes.

(16) The supernate was neutralized with concentrated NaOH and evaporated to 10 ml (inorganic ^{32}P). (a)

(17) From both samples, three 1-ml aliquots were evaporated to dryness in individual planchets.

(18) Each planchet was then counted in the same manner as the previous sample.

(19) One of the remaining two samples of 85 ml was centrifuged at 1250 rpm for 10 minutes. The supernate was decanted and saved (inorganic ^{32}P) (a). The residue was discarded.

(20) These samples were also neutralized with 5 ml concentrated NaOH.

(21) Both samples were evaporated to a volume of 25 ml.

Column 8

(13) One of the 110-ml subsamples was centrifuged at 1250 rpm for about 15 minutes. The organic residue in the centrifuge tube was discarded. The supernate (inorganic ^{32}P) was saved.

(14) Both of the samples were neutralized with concentrated NaOH.

(15) The samples were next evaporated to approximately a 25-ml volume.

(16) Then, three 1-ml aliquots from each sample were dried on planchets.

(17) The planchets from each sample were counted three times for 10 minutes each and the counts were averaged.

The sediment residues remaining after the extraction procedure were dried on planchets. These planchets were counted using the same internal proportional counter. All of the residues contained background levels of ^{32}P. Thus, the extractions were successful in stripping the residue of the ^{32}P.

The results using the Mehta procedure (Table 11) indicate that the ^{32}P was in both the organic and inorganic phases. The fraction of ^{32}P associated with the organic material was by definition equal to

TABLE 11

RESULTS OF THE MEHTA EXTRACTION FOR PHOSPHORUS FROM COLUMNS 4, 6, AND 8

Column Number	Layer	Total ^{32}P cpm*/gm	Inorganic ^{32}P cpm*/gm
4	0 to 1 cm	32,750	19,000
	1 to 2 cm	15,625	13,750
6	0 to 1 cm	3,100(a)	BKG(a)
		29,075(b)	15,925(b)
	1 to 2 cm	1,500(a)	BKG(a)
		11,250(b)	10,125(b)
8	0 to 1 cm	22,950	7,625
	1 to 2 cm	10,550	6,725

* The counts per minute are not corrected for decay.

the total ^{32}P minus the inorganic ^{32}P. This data column was deleted since it was a calculated value whereas the others were measured. In all three columns, most of the activity was found to be in the top layer, i.e., 0 to 1 cm. Within this layer, approximately equal amounts of organic ^{32}P and inorganic ^{32}P were present. However, the lower layer, i.e., 1 to 2 cm, contained almost no organic ^{32}P.

The procedure for column 4 and column 8 differed only as to when the samples were neutralized. Column 4 was evaporated to a 20-ml volume, then neutralized. Column 8 was neutralized first, then evaporated.

For column 6, it was desired to see if much of the ^{32}P was lost through volatilization or sorption onto the walls of the beaker during evaporation of the larger sample. Thus, a 25-ml aliquot was pulled out of the larger volume, and the counts were found to be much lower in this small aliquot than in the larger aliquot. The 25-ml aliquot was apparently too dilute with respect to the total quantity of ^{32}P in solution and thus not representative of the sample. Some sorption of ^{32}P to the evaporating dish is also indicated.

From the analyses completed on the eight sediment samples, the Mehta procedure seemed to be the most reproducible and most amenable to analyzing the sediment for gross ^{32}P fractionation. Other analyses which could possibly warrant future study were the weight comparison and the chloroform extraction procedures. These would require a statistical comparison before the results would be meaningful.

The Mehta procedure indicated that some mineralization of the particulate organic ^{32}P at the sediment-water interface did occur.

Again, a statistical comparison of the results from several columns operated in a similar manner would be necessary before any definite statement could be made.

In comparing these results with the ^{32}P detected in the effluent during the experimental run lasting 45 days, very little of the initial algal ^{32}P trickled into the effluent. The counting rate data were not corrected for decay.

CHAPTER VI

CONCLUSIONS

This research considered the feasibility of a simulated sediment and water system to study the degradation of the particulate organic ^{32}P components of algae, as well as its overall application to the study of sediment-water interactions. The new field of environmental science must develop closely with the advances in engineering research and methods in order to find solutions to many of our environmental problems. This dynamic simulation of the sediment-water interface may provide a research tool for studying sediment-water interactions as related to the movement or cycling of pollutants from the sediment to the overlying water. Though the apparatus described in this research was not amenable to process description (mathematically) for reasons discussed earlier, it does provide insight as to the future development of mathematical models. The releases of Fe and Mn, as well as the reduction of sulfate and trends of the electrode potential and pH, were all predictable in this model. The responses of these parameters were very characteristic of what is known about natural sediment-water systems today. Likewise, the responses between comparable columns were similar. Therefore, models could be used to describe the sediment as a dynamic ecosystem with the lake supplying the necessary source of energy.

It is the opinion of this investigator that the lake ecosystem cannot be adequately understood unless there is a parallel development of our understanding of sediment and its role in the lake ecosystem.

In many of our present impoundments, there has been a tremendous buildup of sediment which has resulted in the impoundments being filled in at a more rapid rate than predicted. Likewise, many sediments in our natural freshwater lakes have been found to contain larger concentrations of nutrients and pollutants than the overlying water. Physical modeling of these sediment-water systems could indicate how these pollutants might be released and possibly methods by which the processes could be controlled.

The algal particulate organic phosphorus fraction of the algal cells would be most resistant to degradation as the cells settled out of the overlying water. Hence, this form of phosphorus was considered to be the predominant natural input to the sediment heterotrophic community. Whereas many investigators postulate that this form of phosphorus would be mineralized and released by the heterotrophic microorganisms in the sediment to the overlying water, very few investigators have studied the length of time for this process to occur. Hayes and Phillips (1958) suggested that the turnover of this phosphorus form was long compared with that of other forms. Phillips (1964) also inferred a long turnover time. Pomeroy et al. (1972) emphasized that the release of phosphorus from the sediments to the overlying water was primarily biologically mediated; direct equilibrium between the sediments and overlying water was of secondary importance. It was quite easy to assume that the mineralization and release of this form were slow, but apparently no one verified how slow. However, Stumm and Morgan (1970) and other investigators suggested that, as the organic phosphorus form was mineralized, the

processes of diffusion in the sediment interstitial water would aid in the interchange processes.

From the trends noted in the data of the present investigation, the availability of the ^{32}P to the overlying water was predominantly biologically mediated. That is, the available mineralized phosphorus in the sediment was sorbed onto the clay particles or precipitated as a metal or calcium compound and was not readily released to the overlying water. From the ^{32}P fractionation attempts, the Mehta procedure clearly indicated that there was a mineralization of algal organic ^{32}P to inorganic ^{32}P in the sediments. Some isotopic exchange with this sorbed ^{32}P was also evident from the data in run 2 after increasing the phosphorus concentration in the overlying water of columns 2, 4, 6, and 8. However, the quantity of ^{32}P released from the sediment was negligible (under all of the imposed environmental conditions) considering the total ^{32}P activity of the algal residue initially added to the columns. The degradation process of this phosphorus form was much slower than previous investigators had considered. For example, the total ^{32}P activity in the extracted algal sample during run 2 was approximately 6,000,000 dpm. Approximately 400 to 500 cpm/2 ml (10,000 cpm/50 ml) was detected in the effluent each day for 45 days. Therefore, less than 10 percent of the total ^{32}P activity was detected in the effluent during the entire time period. Based on this, it would take about 450 days for the exchange on phosphorus from the sediment to the overlying water, if the phosphorus release were regulated by the same processes up to this time.

The release of ^{32}P from days 8 through 16 in most of the viable

sediment-water columns was closely associated with the release of iron and manganese. Since there was not a sustained Fe, Mn, or ^{32}P release after this in most of the columns, it is quite possible that only the surface of the sediment was reduced by biological activity during the experimental runs. The capacity of the sediment to sorb phosphorus, as well as the redox condition of the simulated sediment and hypolimnion, also affected the interchange of sediment phosphorus to the overlying water. Therefore, the initial release of redox-sensitive sediment nutrients, as the hypolimnion of a freshwater lake went anaerobic, would be the largest slug contribution of sediment nutrients to the overlying water during the entire stratification period. Very small amounts of sediment nutrients may continually be added to the overlying water as they become available through degradation of fresh organic material, diffusion, or slight turbulent mixing.

Between spring and fall turnover, the sediment of fairly shallow lakes (characteristic of southwestern impoundments) would be mixed with the overlying water. Consequently, the nutrients sorbed on the surfaces of sediment, as well as the partially degraded organic matter, would be resuspended in the water. Significant aerobic decomposition of organic matter and uptake of sorbed nutrients by the plankton could occur prior to the settling of the material. As a result, a certain quantity of sediment nutrients would become available to the suspended biota and the overlying water in a short period of time.

The results obtained from this investigation emphasized the important role that the sediment heterotrophic community has in the interchange of nutrients, specifically phosphorus, from the sediment to

the overlying water during summer stratification. It is during the summer stratification period that the sediment acts as a "sink" for phosphorus being deposited from the overlying water.

The processes involved in nutrient regeneration from sediment need closer investigation as to the specific mechanisms involved in nutrient turnover and release. Even though the sediment of lakes acts as a "sink" for nutrients, it was quite apparent from this study that at least some of these nutrients may be recycled to the overlying water and not "lost" to the sediment.

The limited field investigation, together with the laboratory model, clearly indicated that the sediment community of organisms was quite stable in spite of the changes in the water temperature, nutrient concentration, and degrees of anaerobiosis. Most important, however, was that the laboratory sediment-water system responded quite reproducibly and predictably to changes in the aquatic environment above the sediment.

Several improvements in the simulated sediment-water system became apparent during this investigation. The apparatus was very time consuming to set up. The reason for this was that the system had to be sealed to prevent seepage of water from the constant temperature jacket into the cultivation chamber. Since the water volume to surface area ratio had negligible effect on the performance of the laboratory system, a sediment core sample with a much larger surface area should be used in order to minimize possible wall effects. By so doing, it would be possible to sample the water and sediment periodically in the cultivation chamber. More accurate chemical and

biological measurements could then be made in situ with monitoring equipment to establish the status of the system. Successional heterotrophic populations could also be studied with little disruption to the overall sediment-water system. Most important, replicate core samples could be studied simultaneously in the laboratory while field investigations were being carried out. By comparing the data, it could be feasible to develop a predictive mathematical model of sediment-water interactions.

Possible applications of this type of experiment could be made in studying the successional biota and water-quality changes attributed to dredged material disposal sites. The biota succession would primarily be concerned with microorganisms establishment, since larger organisms could not be adequately represented. Furthermore, this experimental system was a process model whereby only qualitative information could be obtained regarding phosphorus transformation rates. This type of data could possibly indicate significant trends, etc., which should be studied in detail. This could be investigated by placing dredged material into an apparatus similar to the one described only with a larger sediment surface area in order to sample the organisms. By flowing natural unfiltered water over the dredged material at realistic hydraulic residence times, it would be possible to observe the short-term establishment of biota as well as the possible changes in water quality resulting from biological activity in the dredged material. This type of study could apply in freshwater, saltwater, or estuarine environments. Likewise, this laboratory system

could be adjusted to simulate a stream-sediment or river-sediment system with appropriate adjustment of the HRT.

Just as this apparatus has shown that it was stable and predictable in simulating certain aspects of sediment-water interchange processes, it could also be used to study methods of controlling the release of chemical constituents from the sediment. For example, in considering dredging operations on navigable waterways, the problem of dredged material stabilization at the disposal sites could be investigated quickly for many of the sites prior to the dredging operations. By placing dredged material in a continuous-flow apparatus, it would be possible to add benthic algae at a metered rate and observe the rate at which they become established, as well as any associated water quality changes.

The main advantage to this system for studying sediment-water interactions could be in the qualitative study of chemical interchange processes. By tagging clays, biota, etc., with radionuclides of metals, it would be possible to follow the extent and time required for these materials to be released to the overlying water. This study could involve effects of temperature, pH, mixing, etc. Ideally, the study of pesticide degradation at the sediment-water interface could be performed using a radiolabelled pesticide and appropriate monitoring equipment.

In general, this system could be considered as a very useful laboratory research tool to study "trends" in the aquatic ecosystem using radioactive labels, where possible, and other analytical equipment. Biological studies, as well as chemical interchange studies,

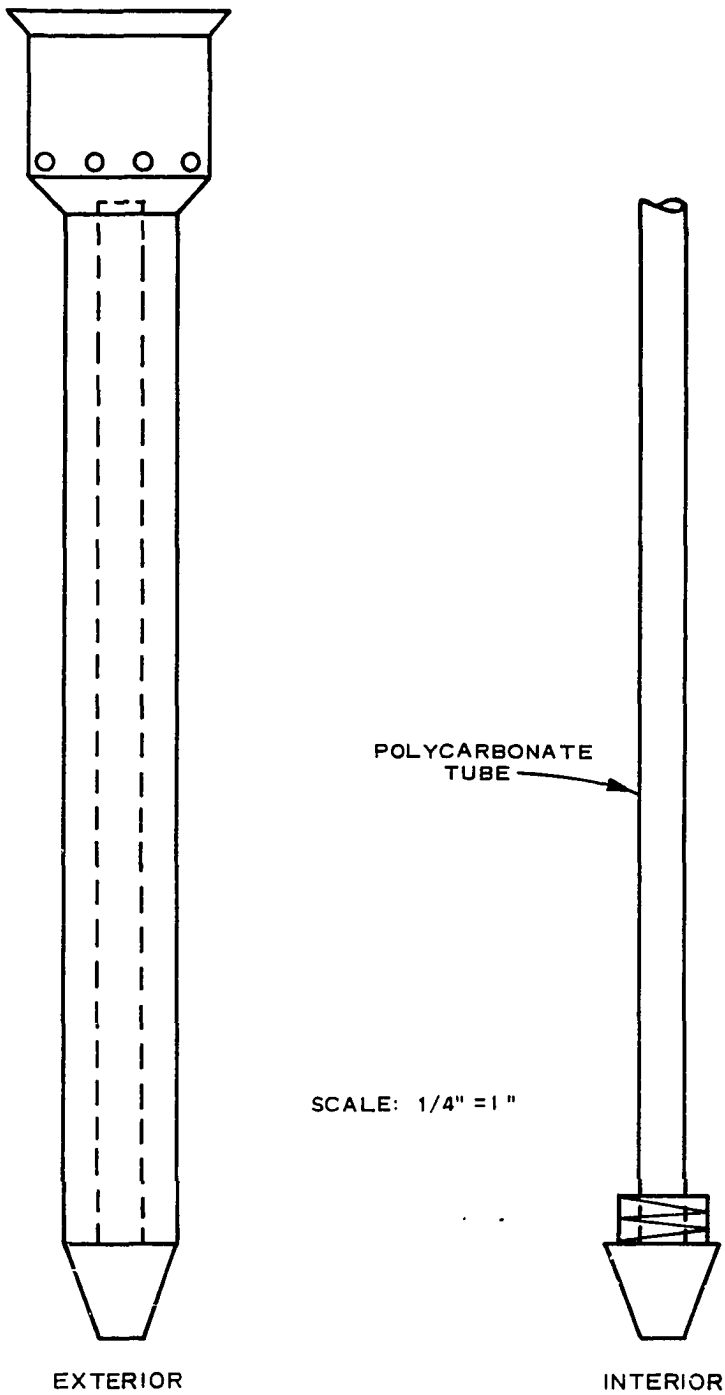
could be performed in the laboratory under controlled conditions while maintaining a realistic sediment-water environment.

Sediment-water interactions must be investigated both qualitatively and quantitatively in the field as well as in the laboratory. The importance of these processes has been well acknowledged. Since most investigations have been empirical and not directly studied, there was felt a need by this investigator to attempt to devise a system whereby a better understanding of these processes could be obtained. The experimental system was considered to be a process model of the sediment-water interface. Through the use of experimental systems such as the one described, a better understanding of the sediment's role in aquatic ecosystems could be obtained.

This research, using a simulated sediment-water system, could be a valuable research tool based on the results of this study. Just as this apparatus has shown that it was stable and predictable in simulating certain sediment-water interchange processes, it could also be used to study methods of controlling certain sediment-water interactions.

APPENDIX A

APPENDIX A



SEDIMENT CORE SAMPLER

APPENDIX B

APPENDIX B

ALGAE AND BACTERIA CULTURE MEDIA

Modified KNOP's Media (Algae)

Solution	Concentration	Amount per liter
tris buffer (trix hydroxy methyl amino methane)	15 g/ℓ	10 ml
vitamin pill (Upjohn Unicap M with minerals)	1 pill per liter	1 ml
EDTA	500 mg/ℓ	1 ml
P	5.62 g K_2HPO_4/ℓ ~1 mg P/ml	(see text)
N	12.1 gm KNO_3/ℓ ~2 mg/ml	5 ml
tryptone $FeCl_3$ $CaCl_2$ $MgSO_4$		trace

Sodium Casienate Broth (Agar) (Heterotrophic Bacteria)

	Concentration	Amount per liter
glycerol	0.2%	2 gm
sodium casienate	0.1%	1 gm
peptone	0.1%	1 gm
starch	0.1%	1 gm
liver extract	0.1%	1 gm
$MgSO_4$	0.01%	1 gm
$CaCl_2$	0.01%	1 gm
$FeCl_3$ or $FeSO_4$	trace	trace
15 γ B_{12}	1 ampoule	1 ampoule
Bacto-Agar		15 gm

After autoclaving, adjust pH to that of the sediment using K_2HPO_4 and/or K_3PO_4 .

APPENDIX C

APPENDIX C

BACTERIA CONCENTRATION (For Top 5 cm)

Run	Average Number of Colonies Aerobic	Average Number of Colonies Anaerobic	Sediment Weight	Bacteria Concentration (Number of Colonies/gm)	Percent Carbon
1	209	54	4.2623	7.63×10^5	9.45
	181	42	4.4618	6.24×10^5	7.77
	291	0	4.9015	7.57×10^5	5.86
	293	139	5.5429	9.94×10^5	7.08
	275	99	5.0135	9.35×10^5	7.31
				8.15×10^5*	7.49*
2	85	48	5.5429	2.93×10^6	10.15
	58	34	4.4610	2.58×10^6	10.36
	105	48	4.7023	3.98×10^6	9.87
	65	45	5.1077	2.96×10^6	10.01
	90	37	5.3075	2.99×10^6	9.17
				3.03×10^6*	9.91*
3	17	1	6.7393	3.34×10^5	7.23
	22	0	6.2985	4.37×10^5	7.78
	11	44	5.2122	1.32×10^6	7.36
	11	31	3.6972	1.42×10^6	8.46
	5	0	10.8093	5.78×10^4	5.84
				7.14×10^5*	7.23*
4	65	35	6.7891	1.84×10^6	7.87
	25	9	5.3275	7.98×10^5	7.23
	22	4	5.8417	5.56×10^5	8.32
	17	2	4.9018	4.84×10^5	7.45
	18	13	6.0100	6.45×10^5	8.51
				8.65×10^5*	7.80*

* Average.

APPENDIX C--Concluded

BACTERIA CONCENTRATION (For Bottom 5 cm)

Run	Average Number of Colonies Aerobic	Average Number of Colonies Anaerobic	Sediment Weight	Bacteria Concentration (Number of Colonies/gm)	Percent Carbon
1	237	227	5.5119	1.07×10^6	6.72
	215	255	5.0148	1.17×10^6	8.00
	257	0	4.9780	6.42×10^5	5.38
	303	189	5.8091	1.08×10^6	7.96
	218	209	4.6375	1.15×10^6	6.89
				1.02×10^6*	6.99*
2	83	45	5.1191	3.13×10^6	9.95
	80	20	5.0301	2.48×10^6	8.48
	80	57	4.8773	3.51×10^6	9.01
	94	84	5.3091	4.19×10^6	9.72
	65	34	4.9015	2.52×10^6	8.94
				3.17×10^6*	9.22*
3	15	0	9.3433	2.01×10^5	7.57
	58	43	6.5357	1.93×10^6	7.60
	24	7	4.9290	7.86×10^5	6.96
	15	1	4.0105	4.99×10^5	7.65
	29	28	7.0571	1.01×10^6	6.40
				8.85×10^5*	7.24*
4	57	6	5.0177	1.57×10^6	7.14
	53	14	5.2138	1.61×10^6	6.81
	24	2	4.7988	6.77×10^5	7.01
	14	1	4.4376	4.22×10^5	7.58
	13	1	4.9831	3.51×10^5	7.22
				9.26×10^5*	7.15*

* Average.

APPENDIX D

APPENDIX D

CHEMICAL QUALITY OF WATER FLOWING TO THE COLUMNS

I. Experimental Run 1	Days 0-14	14-28	29-36	37-50	51-60
A. Lake Water:					
Total dissolved solids (mg/ℓ)	200	200	200	230	220
Eh (mV)	240	260	260	220	240
pH	7.8	8.0	7.7	8.2	8.2
Sulfate (mg/ℓ)	20	16	18	16	18
Ammonia-N (mg/ℓ)	0.15	0.20	0.10	0.15	0.10
Iron (mg/ℓ)	<0.10	<0.10	<0.10	<0.10	<0.10
Manganese (mg/ℓ)	0.10	<0.05	<0.05	0.10	0.10
Total Phosphorus (mg/ℓ)	0.01	0.01	0.01	0.01	0.01
B. Distilled Water:					
Total dissolved solids (mg/ℓ)	0	0	0	0	
Eh (mV)	300	330	330	380	
pH	6.8	7.0	7.0	6.9	
Sulfate (mg/ℓ)	0	0	0	0	
Ammonia-N (mg/ℓ)	0	0	0	0	
Iron (mg/ℓ)	<0.10	<0.10	<0.10	<0.10	
Manganese (mg/ℓ)	<0.05	<0.05	<0.05	<0.05	
Total Phosphorus (mg/ℓ)	0	0	0	0	

II. Experimental Run 2 (Column 5-8)	0-15	16-30	31-45
A. Lake Water:			
Total dissolved solids (mg/ℓ)	190	200	200
Eh (mV)	220	210	240
pH	8.2	8.1	8.1
Sulfate (mg/ℓ)	17	16	16
Ammonia-N (mg/ℓ)	0.18	0.10	0.15
Iron (mg/ℓ)	<0.10	<0.10	<0.10
Manganese (mg/ℓ)	<0.05	<0.05	<0.05
Total Phosphorus (mg/ℓ)	0.01	0.01	0.01

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