THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE EXCHANGE AND GROWTH POTENTIAL OF PHOSPHORUS IN ALGAE CULTURES

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

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Norman, Oklahoma

1970

THE EXCHANGE AND GROWTH POTENTIAL

OF PHOSPHORUS IN ALGAE CULTURES

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ACKNOWLEDGMENTS

The author is indebted to numerous people for assistance in this study.

He wishes to thank Dr. James Robertson, chairman of the dissertation committee, whose supervision was extremely beneficial. Gratitude is expressed to Professor George Reid, for financial support and for his instruction and knowledgeable advice. Thanks are due Dr. Edwin Klehr for his guidance and advice throughout all phases of the study. The comments of Dr. John Lancaster were appreciated as was the experimental assistance of Dr. Ren-deh Tuan. The author is particularly grateful to Sister Mary Clodovia for her help, innovation and inspiration. The author expresses his deepest thanks and appreciation to his wife, Anne, for her encouragement, understanding and typing of the manuscript.

The Atomic Energy Commission is gratefully acknowledged for sponsoring the Research Participation Program for College Teachers, enabling Dr. Tuan and Sister Clodovia to take part in the investigation.

This project was supported by the Office of Water Resources Research, State of Oklahoma, A-011.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

THE EXCHANGE AND GROWTH POTENTIAL OF PHOSPHORUS IN ALGAE CULTURES

INTRODUCTION AND PROBLEM DEFINITION

Eutrophication is no longer thought of as a long-term naturally occuring enrichment process, but rather as a fertilization process greatly accelerated by man and his activities that endangers our lakes, streams and estuaries. In contrast with more publicized water pollution problems such as oil spills and contamination by industrial waste effluents, eutrophication is not characterized by death, immobilization or other immediately noticeable, adverse physiological effects on the inhabitants or consumers of our waters, but rather by the excessive growth and activity of planktonic, photosynthetic vegetation, namely algae.

Algae are particularly troublesome from two viewpoints. In public water supplies many species give rise to taste and odor problems while others interfere seriously with filtration procedures. In lakes or reservoirs, the most serious problems are concerned with their production of nuisance conditions. These are due mainly to the ability

of gentle winds to concentrate algae along the leeward shores where they interfere with recreational activity and often decompose to produce obnoxious odors. Under extreme conditions algal decomposition may decrease oxygen levels in localized areas sufficiently to cause fish kills. Certain algae also secrete toxins capable of killing livestock or other consumers.

Despite the numerous research projects and field studies directed toward an understanding of the enrichment and biological production processes in lakes, streams, and estuaries, there is still little insight into methods of controlling or decelerating eutrophication and the resultant algal growth. The reason for this lack of insight is becoming readily apparent as research proceeds. That is, there is no simple relationship between eutrophication and the amount of algae that will result from a given level or rate of eutrophication. A myriad of environmental variables and interactions among these variables exists and any attempt to predict or correlate the subsequent behavior of a body of water becomes extremely difficult. As illustrated by Rawson (1) even the apparent inter-relationships among the more obvious climatic, physical, chemical and biological factors affecting the metabolism of a lake are very complex (Figure 1).

In view of this complexity it is obvious that a logical approach to a clearer understanding of the behavior of a

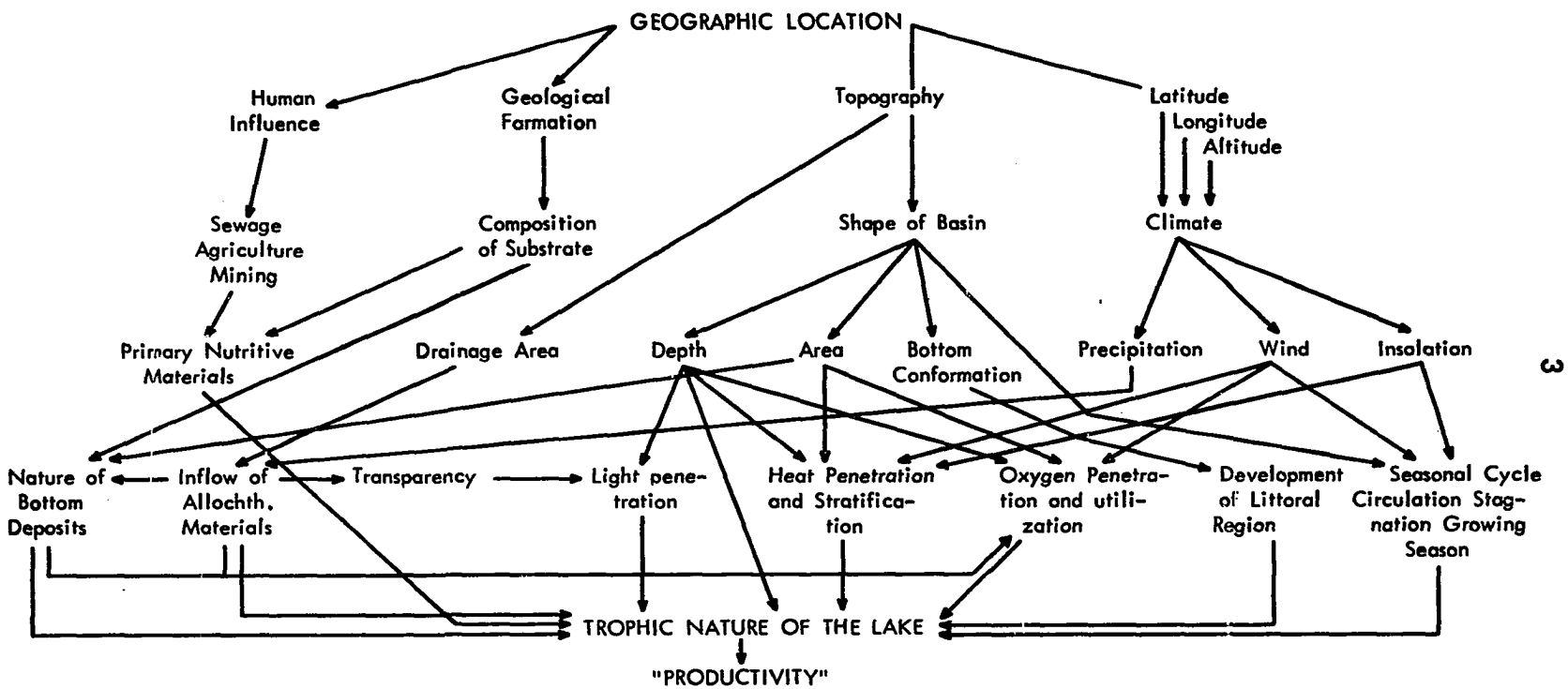


Figure 1. Interrelationship of Factors Affecting Lake Metabolism.

body of water interacting with the surrounding environment is needed. The simplest approach might be to describe or define the most obvious changes in terms of variables or interactions which readily lend themselves to examination and, at the same time, are of primary importance in the overall behavior of the system.

Because excessive algal growth is the most deleterious and obvious effect of eutrophication, it seems appropriate to concentrate on understanding and controlling the associated factors. According to the work of numerous investigators, (Gates and Borchardt, (2); Oswald and Golueke, (3); Fruh, Stewart, Lee and Rohlich, (4); Mackenthun, Keup and Stewart, (5); Weiss, (6)), one of the most important factors associated with excessive algal growth is the nutrient supply. They suggest that by controlling the nutrient input the algal growth potential of a body of water may possibly be regulated. Although some question the need for nutrient control, there seems to be general agreement that some means of nutrient control to arrest eutrophication is necessary.

Assuming that the primary productivity of a body of water can be controlled by limiting one or more nutrients in accordance with Liebig's Law of the Minimum, the question of which nutrient to limit arises.

Since nitrogen is the most prevalent element in algae with the exception of carbon and oxygen, it would seem to

be the most logical point of attack. However, because in wastewaters or biologically treated effluents nitrogen may occur in the form of ammonium, nitrite, nitrate, and organic compounds, and because the removal of all forms by a single treatment method is impossible at this time, the control of nitrogen is not seriously considered in most situations (7).

There is significant leakage of combined nitrogen through soils and thus appreciable amounts reach ground waters. Although nitrogen in organic forms does not penetrate the soil and the ammonium ion is retained effectively by absorption or ion exchange, both forms are gradually converted to nitrate in aerobic soils. Since nitrates are not retained, they are carried along in the ground water unless removed by growing plants (7).

Further argument against controlling nitrogen to limit eutrophication includes the fact that several blue-green algae, Anabaena, Cylindrospermum, Nostoc, etc., are able to assimilate or "fix" elementary nitrogen (8). Thus, there is no assurance that the reduction of the nitrogen budget will limit the growth of nitrogen-fixing blue-green algae.

In addition, in heavily populated areas where the rate of eutrophication is the greatest, there is an abundance of available nitrogen (NH_3, NO, NO_2, NO_3) in the atmosphere

resulting from nitrogen fixation by automobile engines. During rainstorms, this available nitrogen is dissolved in raindrops and much of it is ultimately deposited in lakes or streams (9). Also, minute, but possibly significant amounts of nitrogen are fixed in the atmosphere by lightning and by cosmic rays.

Phosphorus has been selected by many as the most likely point of attack in applying Liebig's Law of the Minimum to control or inhibit eutrophication because chemical removal methods have been well established, costs are not exorbitant and because it is the only known hope of controlling nitrogen-fixing blue-green algae (7).

The basic reasoning supporting phosphorus removal alone as a means of controlling eutrophication stems from two lines of argument. First, its removal will increase the nitrogen to phosphorus ratio and allow phosphorus to become limiting. Second, the green algae hopefully will so reduce the phosphorus concentration in the aquatic environment that growth of the nitrogen-fixing blue-greens will become impossible.

Macronutrients such as calcium, magnesium, potassium, and sulfur are other elements required in significant amounts for most algal growth. But, since all of these exist in the majority of natural waters in significant concentrations, it is reasonable to assume that their removal at controllable sources would have little, if any, benefit.

Iron, manganese, copper, zinc, molybdenum, vanadium, boron, chloride, cobalt, and silicon are micronutrients necessary for the growth of some or all algae. In eutrophic waters, it is reasonable to assume that these micronutrients are present in sufficient quantity. Because of the small concentrations normally present in wastewaters, except for chloride and silicon, feasible means of reducing the concentration of these "trace elements" other than by evaporation are not available (7).

Any critical nutrient i.e., one whose concentration varies inversely with the degree of primary productivity, is a potential point of attack in nutrient control and application of Leibig's Law of the Minimum to diminish primary productivity. The determination of critical nutrients in any lake or stream can be determined by chemical analysis of the water at frequent intervals over the period of a year or by in situ studies during the normal growing season (7). Curves showing typical seasonal variation in the concentration of a critical elements and of noncritical elements in relation to primary productivity are shown in Figure 2. From this type of analysis, phosphorus is often seen to be a limiting factor in lakes (9).

The thought that phosphorus is frequently a limiting factor of algal growth in nature is supported by many authors. Among them are Kuhl, (10); Talling, (11);

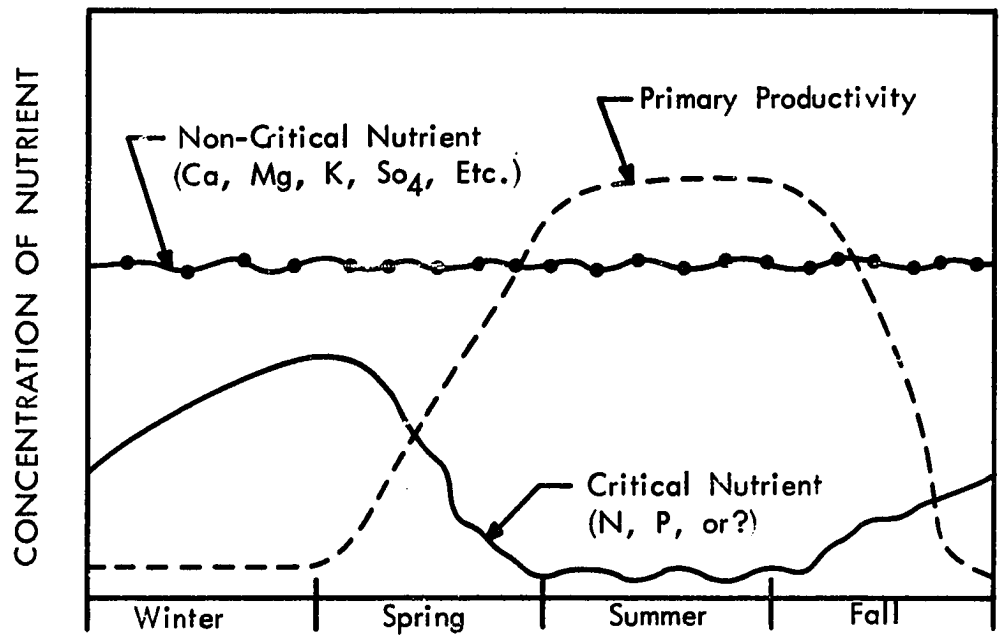


Figure 2. Relationship of critical nutrient concentration in lake waters to primary productivity.

Yentsch, (12); Ketchum, (13); and Wiebe (14). Further, according to the work of several other investigators (Borchardt and Azad, (15); Sawyer, Lackey and Lorenz, (16); Hasler, (17); Smith, (18); Ohle, (19)) the phosphorus content of wastewaters is the most important factor related to excessive enrichment and the resultant algal growth.

In view of this and much other published evidence it seems reasonable to assume that the removal of phosphorus or the simple dilution of the phosphorus to a low concentration will prohibit the stimulation of the ecosystem and that subsequent eutrophication will be suppressed. Before this approach can be effectively used, however, an answer to the following question is needed: If a quantity of phosphorus is present in, or is added to the aqueous environment, can the response in terms of algal growth be predicted? In order to fully answer this question, a great many factors would have to be considered. Included would be the minimal phosphorus concentration necessary for algal growth, the mechanisms involved in phosphorus uptake and assimilation and the effect of factors such as light, temperature and pH on the mechanisms. However, the solution might be more easily attained by simply assuming that the extent of algal growth is primarily a function of the portion of the phosphorus input which becomes available for uptake, assimilation and growth.

It is well documented from bacterial enzyme studies (20) and from sewage treatment studies (21) that the rate of substrate uptake, V, or growth rate, as the case may be, is dependent on the substrate concentration, S, as described by the Michaelis Menten expression:

$$V = S\ V_{max} / (S + K_m)$$

where V_{max} is the maximum uptake rate and K_m is the substrate concentration at which the uptake rate is half-maximal. A study conducted by Hanton and Kuenzler (22) has shown that the relationship between marine planktonic algae and phosphorus concentration follows this expression (Figure 3), and it is reasonable to assume that the same relationship should exist between freshwater planktonic algae and the phosphorus concentration.

Phosphorus exists in many inorganic forms, ortho-, poly-, meta-, and pyro-, and in countless organic compounds. The literature is confusing and inconsistent in categorizing phosphorus compounds. Watt and Hayes (23) classify phosphorus in a simple but descriptive manner and one which will be used throughout this study. They refer to the various compounds as dissolved inorganic phosphorus, DIP; particulate phosphorus, PP; and dissolved organic phosphorus, DOP. DIP refers to the soluble inorganic fraction that is capable of being taken up by plants and bacteria. PP refers to phosphorus that is sorbed onto a colloid or

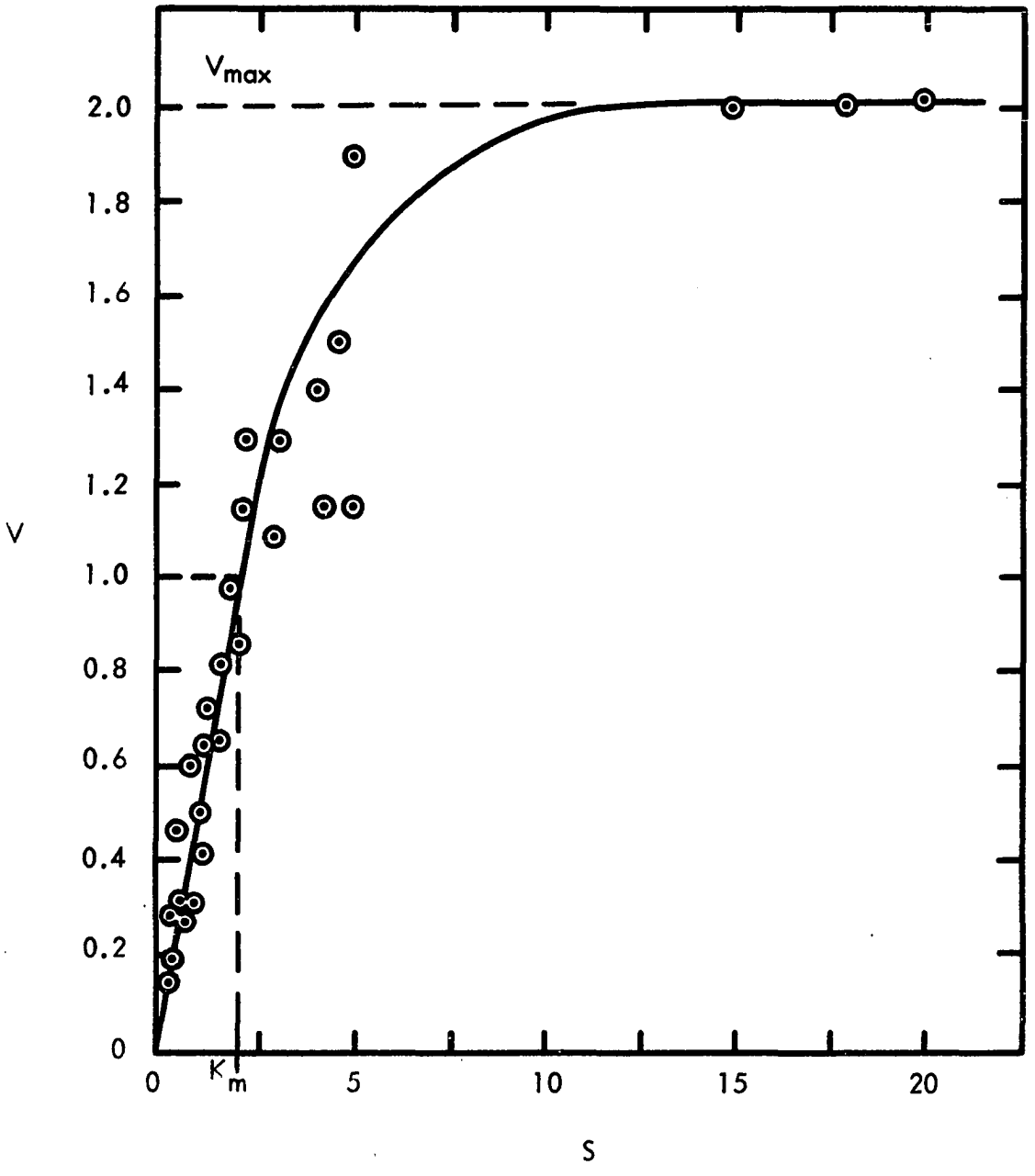
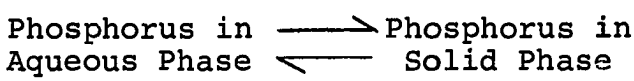


Figure 3. The Uptake Rate, v, at Phosphate Concentrations, s.

cell surface, stored in a cell, or incorporated into intercellular compounds. DOP refers to the soluble organic fraction, some, or all of which, may be ultimately converted to the DIP form by bacteria. Although there isn't complete agreement as to which of the mentioned inorganic forms are available for phytoplankton uptake, the accepted documentation is that the most assimilable form is orthosphosphate (24). Galloway and Krauss (25) found that Chlorella pyrenoidosa growing actively in various polyphosphates changes the varied polymers of phosphate to orthophosphate before absorption. Reid (26) agrees by saying that bacteria take up large quantities of inorganic phosphate either by assimilation into their own bodies or by conversion to the organic fraction thus making the nutrient unavailable for use by green plants. In view of these statements, therefore, it was concluded that in this study the available form of phosphorus was orthophosphate, also referred to as dissolved inorganic phosphate, DIP.

It would be a relatively simple matter to determine the amount of available phosphorus entering a body of water and to correlate it with a given amount of algal growth if all, or a constant, known percentage, of the added DIP stimulated new algal growth. This does not appear to be the case, however, as evidenced from the studies of Phillips, (28); Hayes and Phillips, (29); Rigler, (30); and Hayes et. al.

(31). They found when inorganic phosphorus, DIP, is added to a lake as fertilizer nearly all of it disappears in a few days, and large increases in algal concentration did not necessarily follow. By using labeled phosphorus it was discovered that the phosphorus was distributed throughout the water in bacterial and algal populations and in the sediments (28), (30). In view of these results it could be reasonably assumed that the total phosphorus budget in a body of water is distributed between the aqueous phase (dissolved phosphorus) and the solid phase (phosphorus which is sorbed on colloidal or cellular surfaces, incorporated into cellular substances or found in the sediment) and that these two phases are in a state of dynamic equilibrium as represented by the following model:

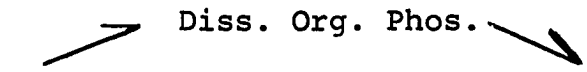


As phosphorus is lost from the aqueous phase it is returned from the solid phase and visa versa. This interpretation does not deny the general observation that addition of fertilizers to lakes stimulates growth. Obviously after equilibration there will be more nutrient in the system than before. The point is that the decline of phosphorus in the aqueous phase is not a direct measure of increased productivity. It seems logical to assume, however, that there may well be a relationship between the amount of phosphorus added, the amount of phosphorus which stimulates

new growth, and the increase in algal concentration.

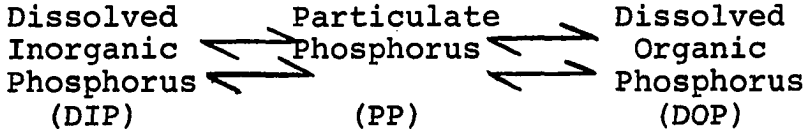
In order to explain this further, one must consider that phosphorus is constantly becoming available for algal uptake from potentially assimilable inorganic phosphorus in the solid phase that has been in some manner sequestered and also from phosphorus which is in another chemical form but is capable of being converted into the available DIP state. Examples of sequestered, potentially assimilable, inorganic phosphorus that may become available for algal uptake include that which is in the sediment and that which is sorbed on the surfaces of cells or colloids. This phosphorus could be made available to phytoplankton at a rate primarily in accordance with the physical, chemical, and biological processes at the mud-water or surface-water interface. The importance of these phosphorus "sinks" has been acknowledged by Gahler, (27); Phillips, (28); and Hayes and Phillips (29). Phosphorus which also must be considered as potentially assimilable, but which is in a form other than orthophosphate is that particulate or organic fraction which is being circulated through the phosphorus cycle and which will eventually be regenerated into DIP by bacterial action.

Diagrams of phosphorus cycles are found in many textbooks, but a very simple one-way phosphorus cycle representing basic phosphorus reactions which might occur in a lake or stream is shown on the following page (23).



The assumption that a one-way cycle such as this is part of a system of dynamic equilibria was strengthened by Watt and Hayes (23) by adding ^{32}P as tracer orthophosphate to lake water and noting its equilibration with the particulate and dissolved organic phases (Figure 4).

By disregarding the additions of phosphorus from the sediments and catagorizing the aqueous phosphorus phase as either dissolved inorganic or dissolved organic, a model, as shown below, representing the distribution of the phosphorus in an aquatic biological system in dynamic equilibrium was developed.



This model constructed by Watt and Hayes (23) may be described and defended in the following manner: The reaction DIP⟶PP represents uptake of orthophosphate by living organisms. Zooplankton do not play a significant role in this reaction. Harris (32) found that ^{32}P uptake by Gammarus was inhibited by antibiotics and concluded that "Gammarus does not take up appreciable quantities of phosphorus by adsorption through the body wall, intestine, or gills." Rigler (33) drew a similar conclusion from his work on

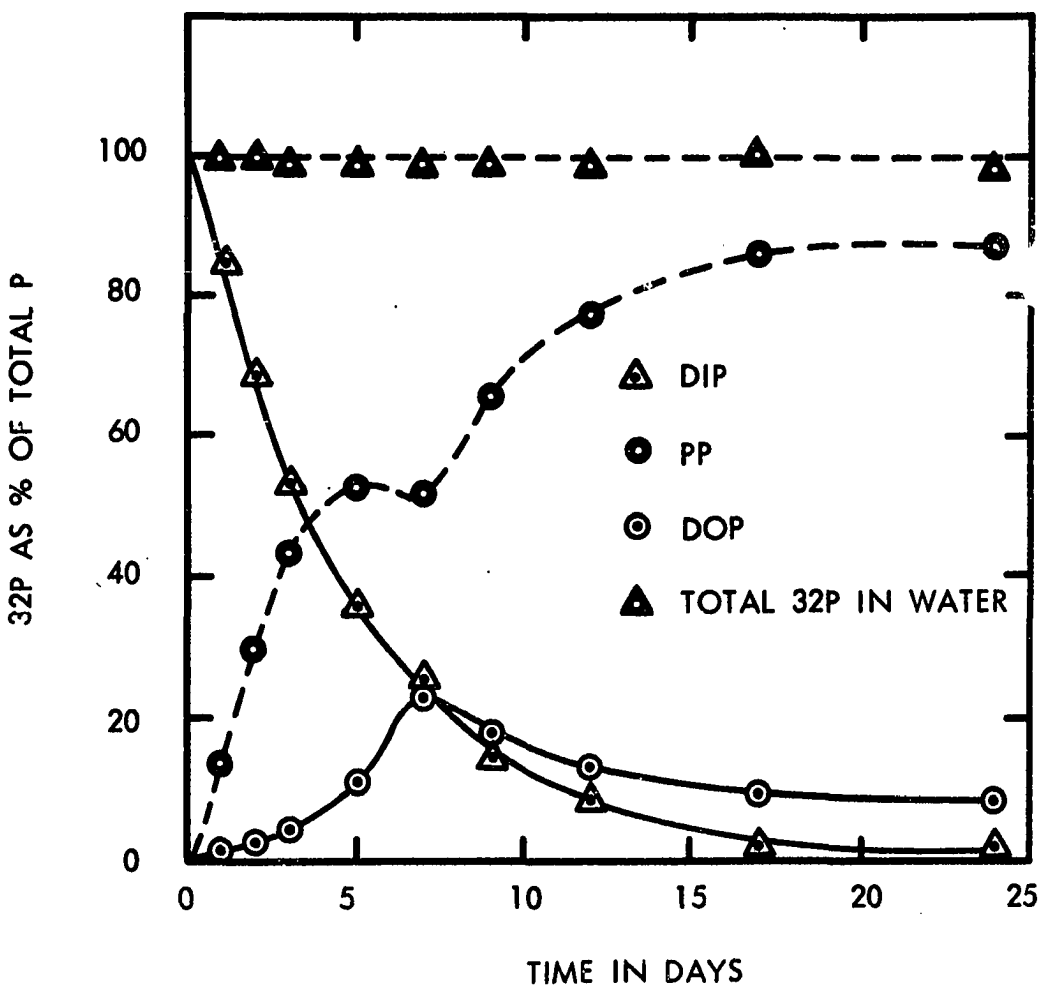


Figure 4. Percentage of DIP-32 Incorporated into PP-32 and DOP-32 with time.

Daphnia magna. Bond (34) has presented evidence that marine invertebrates in general are impermeable to water, salts, and organic solutes.

Harris (32) suggests that bacteria in lake water will take up inorganic phosphate and incorporate it into organic compounds, and the same is doubtless true of phytoplankton.

The process PP $\longrightarrow$ DOP represents the release of organic phosphorus from dead organisms presumably as a result of bacterial attack and leaching out by water.

Hayes and Phillips (29) added tracer ^{32}P to mud-water systems. When antibiotics were used or the systems otherwise sterilized, they found a marked increase in the ^{32}P loss from water to mud. The plants Sphagnum and Eriocaulon also took up more ^{32}P when antibiotics were added. The authors suggest that bacteria take up inorganic phosphorus and return it to the water in an organic form which is retarded from exchange reactions with the sediments and plants, thus allowing it to remain free in solution. They point out, however, that their results can be equally well explained by uptake and storage of ^{32}P in particulate form by micro-organisms in the water.

The return process DOP $\longrightarrow$ PP represents the uptake of dissolved organic phosphorus compounds by bacteria, and possibly phytoplankton and certain protozoans. Krogh (35) in his review of the subject concluded that no multicellular

animals take up dissolved organic substances to any significant extent.

Finally, PP $\longrightarrow$ DIP is the result of several simultaneous processes: (1) the release of DIP from organic compounds which have been assimilated by bacteria; these organic compounds could be taken up from the water or obtained from dead cells, organic detritus, or organic colloids; (2) the return of DIP to the water by phytoplankton and bacteria as a result of exchange in which inorganic phosphate is continually passing into and out of living cells; (3) autodephosphorylation of labile organic phosphorus compounds contained within organisms which have died. Matsue (36) killed Skeletonema cells with chloroform, which leaves the phosphatase active, and by heating, which destroys the enzyme. His results show that stored phosphate, i.e., phosphate taken up by the cells and stored in an organic form, is readily attacked by the phosphatase enzymes in the cell.

Margalef (37) and Rigler (33) have shown that cladocerans release phosphatase into the water of the environment. Rigler found that the filtrate from a bacteria-free culture of Daphnia magna hydrolyzed glycerophosphate dissolved in river water, but naturally occurring organic phosphorus compounds in the same water were unaffected. Rigler concluded that the enzymatic hydrolysis of naturally occurr-

ing organic phosphates does not take place, or takes place extremely slowly.

It may well be that all organic phosphorus compounds subject to enzyme hydrolysis are broken down by the organism's own enzymes as soon as it dies, and only compounds resistant to enzymatic attack are released to the water.

Under steady state conditions this model can be treated as though it consisted of two simultaneously occurring reversible reactions;

$$\text{DIP} \underset{b1}{\overset{a}{\rightleftharpoons}} \text{PP} \underset{c}{\overset{b2}{\rightleftharpoons}} \text{DOP}$$

where "a" represents the rate of phosphorus loss from the DIP phase, "b_1" and "b_2" from the PP phase and "c" from the DOP phase. The calculated exchange rates are applicable only to the particular conditions under which they are determined; they are ecological rather than chemical rate constants.

Assuming that this model is accurate, that it is representative of the total phosphorus budget, and that phosphorus is the limiting factor in a particular situation, valuable insight into algal growth potential may be obtained from its manipulation. For example, the amount of phosphorus in the PP phase under equilibrium conditions is the fraction of the total phosphorus budget that was available for, and is ultimately incorporated into, algal and bacterial cells. The amounts of phosphorus in the DIP and DOP phases reflect

not only the amount of phosphorus not presently incorporated into new growth, but also that which is required for maintaining the system under the prescribed steady state conditions. Within limits, these conditions are probably governed by the amount of phosphorus initially applied to the system. This relationship has been shown previously by application of the Michaelis Menten expression. Thus, a connection between the amount of phosphorus added to a system, the amount actually involved with productivity, and the algal growth potential, seemingly exists.

Proper manipulation of the model will generate values for the exchange rates, a, b_1, b_2, c, which in turn may be useful in providing information pertaining to the time necessary for an unequilibrated system to arrive at an equilibrated state.

The equation, DIP $\longrightarrow$ PP, contained in the model, represents phosphorus uptake. The rate of uptake will follow the Michaelis Menten expression as long as phosphorus remains limiting i.e., uptake is dependent of phosphorus concentration. Accordingly, one should be able to predict the exchange rate, a, from a knowledge of the total amount of phosphorus in the system and the algal concentration; two factors which should greatly influence this exchange rate. The same reasoning should apply toward the reaction, DOP $\longrightarrow$ PP, except that the bacterial concentra-

tion rather than the algal concentration would have to be known. The rates of these reactions influence the reverse reactions and, therefore, it may be possible to predict their exchange rates.

This study then is intended to provide information concerning nutrient control as a means of decelerating the eutrophication process.

More specifically, by using tracer and radiochromatographic techniques, the distribution of phosphorus at varying concentrations in batch algal-bacterial cultures will be determined. It is hoped that this knowledge can be applied to the steady state model constructed by Watt and Hayes (22), and by mathematical manipulation of the model and experimental data that exchange rates and turnover times can be calculated and predicted and, further, that a meaningful relationship between the total phosphorus budget, the amount of available phosphorus, and subsequent algal growth can be established.

LITERATURE SURVEY

In addition to previously noted references, there have been many literary contributions made regarding the role of phosphorus as it pertains to eutrophication and algal growth. Because such problems are being studied by people from various disciplines, a well diversified literature is being produced. Kuhl (38) states that great effort has been concentrated on the subject of phosphorus metabolism since it has been proven experimentally to be connected with the energy transforming systems of living cells and that phosphorylated compounds participate in the reactions of photosynthesis. In general, phosphorus probably affects growth by participating in a number of processes at the cellular level. When these processes are interfered with, secondary changes result, and the metabolic behavior of the organism may be entirely changed. Therefore, it appears mandatory that phosphorus be present in sufficient quantity in order for an organism to behave properly or to exist at all.

Reid (26) states, "In ecological thinking, phosphorus is often considered the most critical single factor in the

maintenance of biogeochemical cycles. This extreme importance stems from the fact that phosphorus is vitally necessary in the operation of energy transfer systems of the cell, and that it normally occurs in very small amounts. The latter factor means that there is apt to be a deficiency of the nutrient, and this in turn could lead to inhibition of phytoplankton increase, resulting ultimately in decreased productivity in the system."

Agreement that phosphorus is often a limiting factor in aquatic environments is voiced by Hutchinson (39). According to him, a comparison of the chemical composition of most freshwaters with that of algal cells suggests that silicon, nitrogen, and phosphorus are among the elements most likely to be depleted by algal growth. He further concludes that in many bodies of freshwater, growth of phytoplankton tends to be limited by the supply of inorganic phosphate. He reports that concentrations of phosphate-phosphorus are often below 10 µg/l, and in some lakes they may be reduced to 1 µg/l or less by uptake during algal growth.

A number of other investigators (Hasler, 17; Maciolek, (41); Mackenthun, (42); Sawyer, (7)) have indicated that the degree of aquatic productivity in a natural water system is influenced directly by the concentration of phosphorus present. It has also been shown that an increase in phosphorus con-

centration results in an increase in the amount of nitrogen fixed (40). Such information indicates the fundamental need for phosphorus control to limit algal growth.

There is little agreement, however, as to the amount of phosphorus that is considered to be limiting. Chu (43) worked with natural waters and found indications that phosphorus concentrations of less than 0.05 mg/l could be limiting for algal growth, while concentrations of 0.1 to 2.0 mg/l orthophosphate-phosphorus could support good growth. Concentrations higher than those found in natural water systems were required for optimum growth rate, but concentrations as high as 20 mg/l were inhibitory. Rodhe (44) measured substance production, cell multiplication, and chlorophyll, and found the limiting concentrations to be less than 0.5 mg P/l. Higher concentrations were inhibitory. Sawyer (45), from some early field work, thought that 0.03 mg P/l was the limiting concentration. Gerloff and Krombholz (46) found that a certain concentration of phosphorus is required to synthesize the protoplasm of phytoplankton. The average phosphorus concentration of aquatic plants studied was determined to be about 0.12 percent of the dry weight. Gerloff and Krombholz stated that this quantity should be approximately the minimum cellular phosphorus concentration required for maximum growth of mixed algal cultures. Lange (47) stated that as little as 0.02

mg/l P. may sustain growth of phytoplankton in natural waters. Reid and Gearheart (48) found the minimum concentration necessary for mixed algal growth in their study was .01 mg/l of PO_4-P or lower.

Nitrogen along with phosphorus is often considered limiting. Chu (49) postulated one reason for a non-successful plan of fertilization for fish growth or for standard growth in culture media is that the concentrations are excessive rather than insufficient. He stated, "The growth of plankton is not markably affected by an increase or decrease of nitrogen or of phosphorus and is independent of the N/P ratio in the solution, as long as these elements remain in optimum concentrations. It is mainly the deficiency of nitrogen or phosphorus but not the N/P ratio, that limits the growth of the plankton studied when the concentrations of these elements are below the optimum range."

All the organisms he studied could be grown in nitrate-nitrogen concentrations from 0.9 to 3.5 mg/l and phosphate phosphorus concentrations from 0.09 to 1.8 mg/l. The growth of algae was limited when the concentrations of nitrogen and phosphorus dropped below 0.1 mg/l NO_3-N and 0.009 mg/l PO_4-P. Concentrations of either nitrate nitrogen cr phosphate phosphorus higher than 45 mg/l produced a marked inhibiting effect on the organisms studied.

Shapiro and Riberio (50) determined that even with

high levels of phosphorus, the addition of secondary wastewater treatment-plant effluent to the Potomac River water greatly increased the growth of both green and blue-green algae in proportion to the quantity of effluent added. Even small percentages of effluent added were effective. They considered PO_4-P the sole component for stimulating the blue-green algae, assuming that these organisms were able to provide their own nitrogen supply through fixation of atmospheric nitrogen. The green algae required both PO_4-P and NH_3-N for stimulation of their growth. Removing the ammonia from the effluent controlled the green algae but only the removal of the phosphate limited the development of the blue-green algae as well.

Kuentzel (51) argues that because the amounts of phosphorus necessary to support massive algal blooms are quite low, the actual determining factor in blue-green algal growth is the amount of CO_2 made available by bacterial decomposition of organic matter.

These conflicting results, as to which substances and how much of a substance is limiting, render definite conclusions impossible. Further deterministic experimentation will be necessary to provide the answers.

Sources of phosphorus include runoff, rainfall and industrial and domestic effluents. Reimold and Aiber (52) analyzed rainwater for inorganic phosphorus and found

concentrations of 5 to 150 µg-atm/l with the low concentrations occurring during the winter months. They believe the high concentrations are due to increased agricultural activity during the summer and may be a significant source of nutrients.

Owens and Wood (53) found in the river they studied that fertilizers used in farming were the main source of nitrogen, but phosphorus came mostly from waste-water treatment-plant effluents. They concluded that approximately 50 percent of the phosphorus in the effluents came from detergents. Missingham (54) estimated the per capita/year contribution of phosphorus to surface waters to be 2.5 lb. (1.1 kg) via waste-water treatment-plant effluents. In the river system he studied, runoff from the surrounding watershed was insignificant in its phosphorus contribution when compared to this source.

The mechanism by which phosphate enters the microorganic cell is not fully understood. Data of Furchgott and Shore (55) indicated possible entry by some physical process. The fact that some phosphate entered the cell at 2°C tends to favor a diffusion mechanism since, at this low temperature, cell metabolism is essentially stopped. Growth-promoting temperatures produced a five to ten-fold increase in phosphorus absorption, thus, according to them, emphasizing the metabolic nature of phosphate uptake. They

neglect to point out, however, that a temperature increase accelerates diffusion.

Kamen and Spiegelman (56) continued the study of the mechanism of phosphorus uptake. They reasoned that if the phosphate absorption mechanism is essentially physical, then chemicals interfering with various steps in the phosphorylation cycle would not interfere with phosphate penetration of the cell. The use of a variety of specific enzyme inhibiting chemicals was found to almost completely stop phosphate uptake through inactivation of phosphorylating enzymes while not otherwise harming the cells. Such results support the theory that phosphate enters the cell as a result of the biochemical mechanism of esterification either within or on the cell membrane or within the cell matrix.

Rice (57) studied the exchange mechanisms in axenic Nitzschia closterium by using radioactive phosphorus. His data indicated that exchange varied with changes in the phosphorus concentration of the medium and with physiological conditions of the cells.

A variety of factors have been noted in the literature as having a distinct effect on phosphorus uptake by microorganisms. A list of such influencing conditions would include light, phosphate concentration of the medium, pH, temperature, nitrate, potassium, sodium, organic compounds, etc.

Uptake of phosphate by phosphorus-deficient cells of

the algae Nitzschia closterium and Chlorella pyrenoidosa was always significantly greater in light than in the dark (13). Uptake of the element by normal cells of Chlorella pyrenoidosa and Scenedesmus D_3 of Gaffron was also accelerated by illumination (58). Uptake of phosphorus by Chlorella vulgaris was accelerated by light especially in the absence of carbon dioxide (59). Light of short duration slightly enhanced the phosphate uptake by cells of Scenedesmus quadricauda; longer periods of illumination (10-120 minutes) increased the amount of absorbed phosphate from 50-320 percent over that incorporated in darkness (60). Batterton (61) found that a rapid uptake of inorganic phosphate occurred when phosphorus-deficient cells of Anacystis nidulans were transferred to a medium of excess phosphorus content in the dark. Data obtained during this rapid dark fixation of phosphate suggested that binding of phosphorus is a specific metabolic process and that approximately 25 percent of the normal cellular phosphorus is possibly bound on these sites. Light had little effect on this first phase of phosphate uptake. Subsequent uptake, however, to normal phosphorus content per cell required light and nitrogen.

The phosphorus content of Chlorella and Scenedesmus cells was shown to be strictly dependent on the concentration of phosphorus in the growth medium (58). This finding was also demonstrated to be true for the diatom A. formosa

(62). When phosphate was supplied in excess, the phosphorus content per cell of Chlorella pyrenoidosa was found to be constant, (63).

The hydrogen-ion concentration or pH of the growth medium may change the phosphate uptake rate either by direct effect on the permeability of the cell membrane or by changing the ionic form of the phosphate (64).

Batterton (61) showed that phosphate uptake is temperature dependent and is retarded by anaerobic conditions. Complete inhibition of uptake was demonstrated by heating or freezing. These findings again suggest that phosphorus uptake is a specific metabolic process.

For Nitzschia closterium, the phosphorus uptake rate was influenced by the nitrate concentration of the growth medium (13). In long-term phosphate fertilization studies on a eutrophic lake, results suggested stimulation of nitrate utilization by phosphate enrichment (65). Data of Abbott (66), however, on respiration and gross oxygen production by algae indicated no interaction effects as a result of simultaneous nitrate and phosphate addition.

Potassium promoted the absorption of phosphate by Chlorella vulgaris for both light and dark conditions (67). Sodium ion was indicated to be a key substance in the phosphate uptake process in an obligately marine fungus (68). Sodium chloride was observed to stimulate respiration and

to be required for inorganic phosphorus uptake by the obligately marine fungus, T. roseum (69). Glucose in the concentration of 0.2 percent was demonstrated to suppress phosphorus uptake by Chlorella vulgaris in light (59). Glycolic acid was observed to accelerate the uptake of phosphorus by A. braunii (70).

A number of investigators have noted the possibility of phosphorus storage by algal cells much in excess of their immediate growth requirements (Franzew, (71); Ketchum, (13); Rodhe, (44); Gest and Kamen, (58); Lund, (72); Al Kholy, (73)).

It has been demonstrated that algae with surplus amounts of stored phosphorus could continue to grow even when the external supply of phosphorus was depleted. A four-fold increase in phosphorus content of Microcystis aeruginosa was observed when the external phosphorus supply was sufficiently high. In cultures of cells transferred from the medium with high phosphorus content to one lacking phosphorus, growth increased approximately 300 percent (10).

Gest and Kamen (58) found that the total phosphate content and its distribution in the cell were strongly dependent on the inorganic phosphorus content of the growth medium. Cells which were grown in a medium rich in phosphorus were able to store an appreciable amount of soluble phosphate which was readily lost when the cells were suspended in water or normal saline solution. The removal of a large portion of

this excess phosphate by washing apparently had no effect on the photosynthetic ability of the cells. This suggests that "excess" soluble phosphate is dispensable to the cell.

Mackereth (62) noted in phosphorus-poor lakes (below 1 µg P/l) that the diatom Asterionella could take up and store phosphorus in reserve. He ascertained, further, that growth could continue in a phosphorus-deficient medium by making use of such reserve phosphorus, the cellular phosphorus being steadily reduced. Mackereth found the limiting requirement of phosphorus per cell to be quite small--approximately 0.06 µg $P/10^6$ cells.

Borchardt (15) found that algae could store large amounts of phosphorus and use it for growth at later times when the phosphorus content was very low. At concentrations above 1.5 mg/l PO_4^{-3} the algae Scenedesmus and Chlorella could take up amounts not needed for immediate growth. Because of this added uptake, he concluded that algae blooms are possible even at extremely low concentrations of phosphate in the water.

The association between phosphorus and eutrophication is mentioned often in the literature, but the exact role of phosphorus as a causative factor has not yet been determined. Kliffmuller (74) compared the concentrations of inorganic PO_4-P over several years in samples taken from Lake Constance. Based on an increase in the concentration of

PO_4-P from 1935, when no dissolved PO_4-P was recorded, until 1958 and 1959 when 7 to 9.5 mg/l of PO_4-P were found, the lake changed from an oligotrophic to a eutrophic condition.

In discussing the role of phosphorus in the eutrophication of water, Ohle (19) considered deep lakes to be more sensitive to an increased supply of nutrients than shallow lakes. Considering the dilution factor this, theoretically, should not be the case. However, as a consequence of stratification which limits the supply of oxygen to the lower waters and permits nutrients to go into solution if anaerobic conditions develop, this may be the situation in some areas. In eutrophic lakes the transformations of phosphorus are coupled with the iron cycle, i.e., by the adsorption or absorption of phosphate by ferric hydroxide. The greatest adsorption of iron by phosphate was found empirically by Ohle to occur at a pH of 5.9. He suggested that since a nutrient such as phosphate is continually taken up by an oxidized sediment, the oligotrophic lake with oxygen throughout is much more stable.

The amount of soluble phosphate in Takasuka Pond, measured by Yoshimura (75), was too low to establish a relationship to the other variations in the pond. The high amounts of total phosphate in the spring did coincide with a rise of phytoplankton.

Field investigations on Lake Wingra in Wisconsin by Tressler and Domogalla (76) seemed to indicate that there was little correlation between the soluble phosphorus and the phytoplankton or between the centrifuge plankton and the organic phosphorus. The soluble phosphorus in Lake Wingra was low in the summer and high in the winter.

Ahlgren (77) found that sanitary wastes from 1,300 people and wastes from a yeast factory are causing Lake Narrviken, Switzerland, to have algae (blue-greens) blooms. Only 40 percent of the nitrogen and 50 percent of the phosphorus were found to leave the lake each year. At low dissolved oxygen concentrations, nitrogen was lost to the atmosphere as a result of denitrification and phosphate was precipitated out at high dissolved oxygen concentrations.

Mackenthun et. al. (42) found that as a result of municipal and industrial wastes entering Lake Sebasticook, Maine, during the last 15 years, large algae growths are starting to appear. The most dominant algae were species of the blue-greens, Anacystis and Anabaena. As a result of these blooms, land value along the shores went down. He estimates that 80 percent of the phosphorus now entering the lake would have to be removed to prevent such excessive growths.

Weiss (6), after studying the relationship of phosphates to eutrophication, concluded that the chemical nature of the

particular phosphorus species, its association with colloidal or particulate materials, and the specific associated phytoplankton all contribute to the rate of utilization of the phosphate pool and thus the rate of eutrophication.

Thomas (78), from an evaluation of eutrophication in central European lakes, notes that it is possible for a lake to have a low spring maximal value of phosphate ions, but because of high daily additions of phosphate it may, nevertheless, show a trend toward eutrophication. He often finds this phenomenon in large lakes to which a lot of phosphates have recently been added. He further concludes that if phosphate addition from the catchment area of Zurichsee Lake were stopped, the growth of algae in the lake would stop as soon as the algae consumed the spring maximal phosphate content.

According to Provasoli (79), the increased availability of nitrogen and phosphorus resulting from runoff and treated sewage should benefit all algae equally and should result in more growth of the normal flora. He, however, modifies this position by saying that, "This increase in growth generally happens in vitro with unialgal bacteria-free cultures. In nature, however, species are never alone, and increases in these nutrients sometimes seems to induce a change in flora. This change is probably due to the tolerances of the various algae and their need for certain levels of N and P, the

differential efficiencies of algae in concentrating the nutrients, and the competitive relations of different species of algae."

One of the earlier investigations using radioisotopes to study the phosphorus cycle in lakes was that of Hutchinson and Bowen (80). Ten millicuries of ^{32}P, made up as sodium phosphate in a solution of sodium carbonate, were added to Linsley Pond, Connecticut, in 24 approximately equal portions. As determined from water samples collected in the deep part of the lake, both an upward and downward movement of phosphorus was indicated. About 1,000 times as much phosphorus was taken up by the plants in the littoral zone as was found in the water itself. Some of the phosphorus was precipitated or sedimented as particulate matter through the dead phytoplankton and the feces of the zooplankton. Hutchinson and Bowen suggested that the plants may compete with the phytoplankton for phosphorus. A steady state concentration by the phytoplankton was thought to have maintained the low concentrations of phosphorus in the lake during the summer.

In an experiment by Coffin et. al. (81), 100 millicuries of ^{32}P were added to the surface of a bog lake near Halifax, Nova Scotia. They found that the isotope did penetrate into some of the deeper water but at no time did it go completely to the bottom. The vertical distribution of the

isotope was attributed to some turbulence and migrating plankton. Sphagnum took up the isotope in two peaks, the first peak between four and ten hours and the second at about three weeks. The first peak was attributed to active cell metabolism and the second to more permanent incorporation of the phosphorus into the protoplasm.

Hayes and Coffin (82) described three experiments involving ^{32}P (100 millicuries) in lakes. In one small but highly stratified acid bog lake there was a rapid uptake (within two hours) of the isotope by zooplankton, sponges, Sphagnum, and algae. The fish failed to show much uptake for several days. In this experiment the isotope did not penetrate below the thermocline. In another experiment ^{32}P was added about one meter above the bottom of the same bog lake. An independent benefit of this second experiment was the observation of physical movement (max. lateral spread of about 1.5 meters/hour) of the water below the thermocline. The vertical movement of the water appeared more restricted. In this second experiment Hayes and Coffin found the phosphorus could be concentrated up to 40,000 times in certain plants and the mud had taken up large concentrations of the isotope.

In the third experiment, a lake, which was ordinarily mixed from top to bottom, had 1,000 millicuries of ^{32}P added to its surface. The activity of the isotope lessened

rapidly. They interpreted this as a rapid exchange and equilibrium between the mud, plants and water.

Hayes et. al. (31) discussed the use of isotopes in tracing nutrients through a lake system. After application of 100 millicuries of ^{32}P to Bluff Lake, near Halifax in Nova Scotia, Canada, Hayes, et. al., observed a continuous exchange of phosphorus occurring between the water and solids of the lake. They use the term solids to include the plants and the animals in the lake, as well as whatever thickness of mud enters into the equilibration. The general turnover time for the phosphorus in Bluff Lake was about 5.4 days. However, for the participating phosphorus in the solids, the turnover time was 39 days. Only one sixth of the total phosphorus involved was contained within the water itself; the rest was in the solid material. Only a thickness of about one millimeter in the mud was considered to be the area of exchange. Therefore, only a small fraction of the total fertilizer could be used as a nutritive agent, the rest being exchanged throughout the system.

Rigler (30) applied ^{32}P to Toussaint Lake, a small bog lake near Chalk River, Ontario. Again in this experiment a large quantity (77%) of the isotope was lost from the water and plankton shortly after application. The turnover time of the phosphorus was estimated to be 3.5 days within the entire epilimnion. The turnover time of the phosphorus in

the surface waters was estimated to be only five minutes, which suggested a very rapid turnover of mobile phosphorus within the system.

Watt and Hayes (23) used ^{32}P to determine the distribution and rates of exchange for phosphorus between the dissolved inorganic, particulate, and dissolved organic phases. Their steady state model has been referred to earlier in this study.

Phillips (28) considered the importance of microorganisms (bacteria) in the exchange of phosphorus between the water phase, the sediments, higher plants and zooplankton. The results of his experimentation suggested there normally exists a competition between the various solid phases for phosphorus in natural waters so that the phosphorus in all phases represents a potential reserve of phosphorus for any one of the living components. He further concluded that microorganisms play an essential role in witholding phosphorus from other solid phases such as the sediment and, thus, make it available to the zooplankton and, hence, to higher groups in the food chain.

Information in the literature concerning the algal growth or biostimulatory potential of enriched waters is scarce. Jewell and McCarty (83) state that "the minimum requirement of a given nutrient has practical significance since if it were known, the maximum yield of algae under

conditions favorable for growth could be estimated and therefore the maximum effect of algae on the environment could be predicted."

Oswald and Golueke (3) presented a simple, inexpensive bioassay procedure to evaluate the algal growth potential of a sample of water. McNulty and Galler (84) applied the procedure to the Pamlico River in North Carolina. Results indicated that although the test is easily run, its reliability is lacking.

Pearson, et. al., (85) attempted to kinetically assess the biostimulatory character of various waters. They determined and compared mean growth rates for Selanastrum gracile in Lake Tahoe water, in water from four Tahoe tributaries, in waste effluents, and in water with nitrogen and phosphorus additions. The results indicated that Lake Tahoe and most of the inflows into the lake were nitrogen limiting. Other results were unusual and prompted the authors to question the credibility of batch-type assay procedures.

EXPERIMENTAL PROCEDURE

When a single component of a complex process such as eutrophication is investigated, the remaining components should be optimized and held constant if possible. In this way, changes in the system can, with a greater degree of assuredness, be attributed to a specific factor, and the system in general can be more accurately described. In this study, comprised of two separate experiments, an attempt was made to optimize all conditions which pertained to growth or phosphorus uptake with the exception of the nitrogen and phosphorus concentrations, which were varied.

Throughout the experimentation the physical conditions, temperature, humidity, light intensity and agitation, were kept constant. The humidity in the culturing room was maintained at close to saturation to suppress evaporation from the culturing flasks. This was done with vaporizer-like devices which maintained the relative humidity between 85-90 percent. Temperature in the culture room was stabilized at 25°C by use of centralized heating and cooling controls.

The light sources consisted of seven 40 watt and six 20 watt "Plant-Gro" fluorescent lights operating on a 14-10 hour light-dark cycle. An intensity of from 225 to 250 foot candles was provided depending upon the geometrical positioning of the culture flask in relation to the lights. The flasks were interchanged in order to provide uniform light intensity to all cultures over a period of time. The culture flasks were agitated to keep the algae suspended and dispersed and to induce more rapid gas exchange between the media and the atmosphere. The culture apparatus is shown in Figure 5.

Algal concentration was determined by the colorimetric method described by Reid and Gearheart (48). A Bauch and Lomb Spectronic-20 set at 580 mu was used to obtain the percent transmittance of an algal solution. To provide a means of conversion from percent transmittance to Areal Standard Units (ASU) or dry weight of algae, a calibration curve relating these three measurements was developed (Figure 6). With the aid of a Whipple eyepiece and a Sedgewich-Rafter counting cell, algal counts corresponding to particular percent transmittance were made. In similar fashion, samples from algae cultures of known transmittance were diluted, dried and weighed, thus correlating weight and percent transmittance.

As was observed by Reid and Gearheart (48) slight

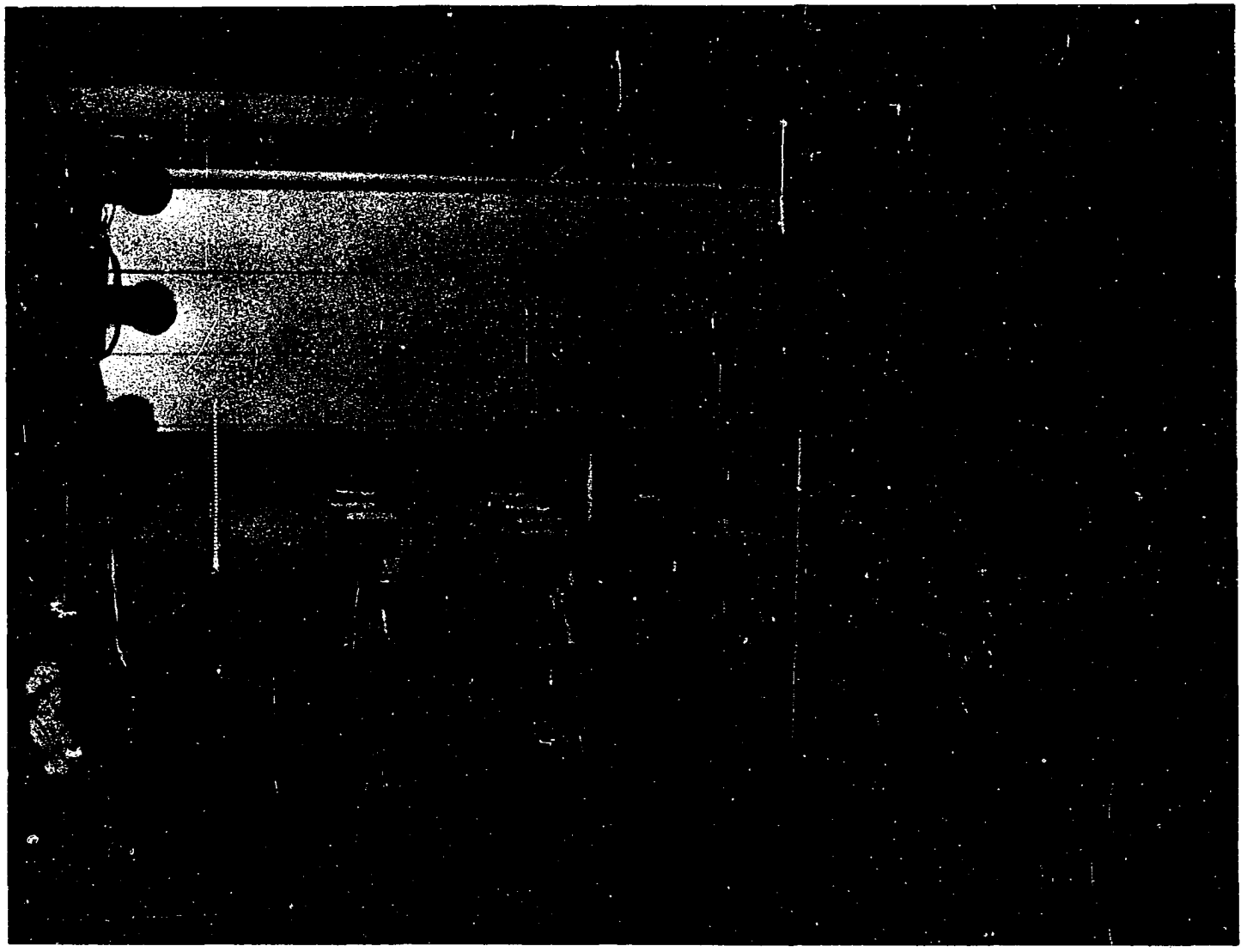


Figure 5. Algae culturing apparatus.

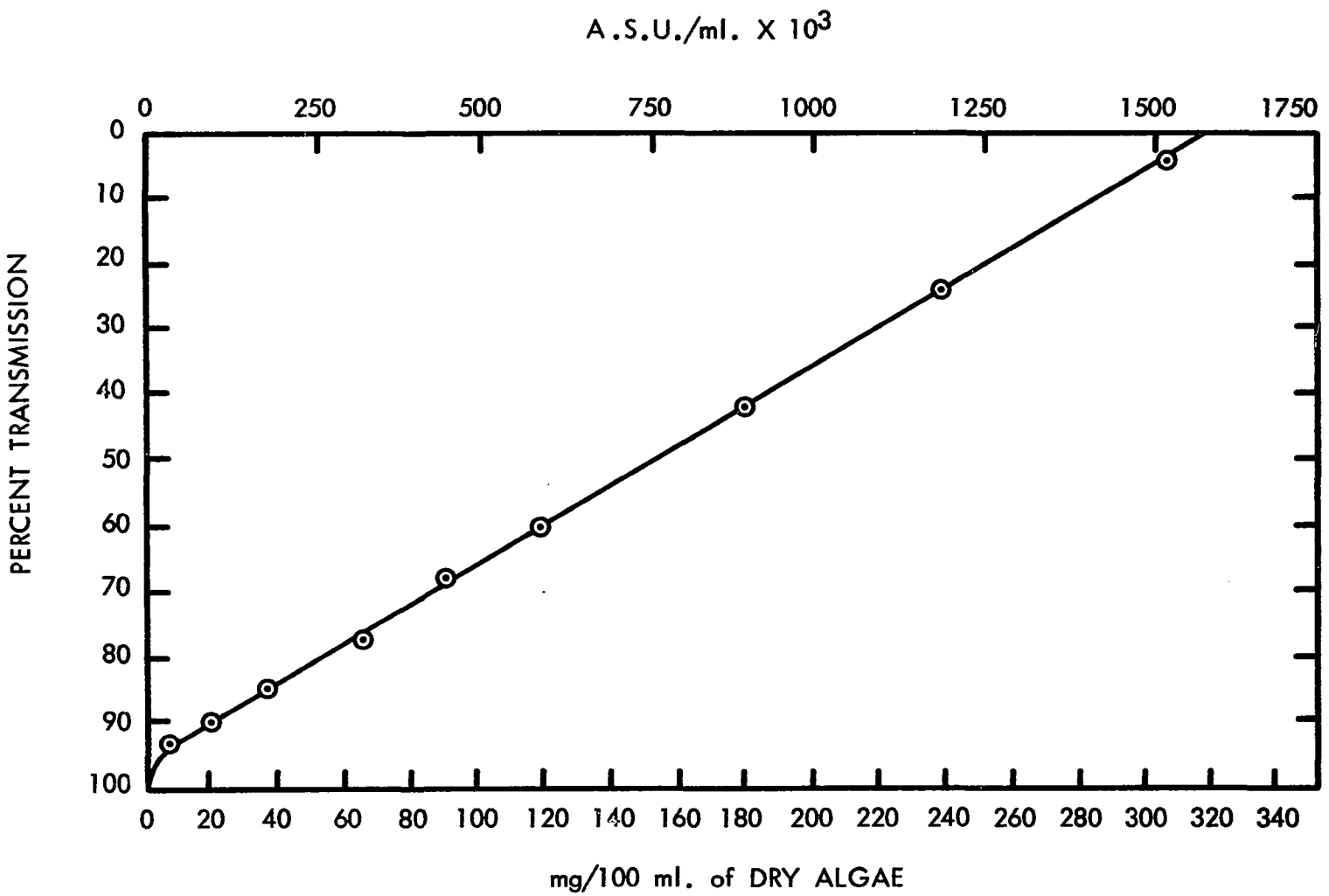


Figure 6. Standard curve for percent transmittance, dry weight, and Areal Standard Units.

variations occurred in the point of maximum absorption in the latter growth stages. This was due to the color shift, green to yellow, in the cells as they aged.

Algae were batch cultured in 250 milliliter Erlenmeyer flasks with cotton plugs in the first experiment. As a result of agitating the flasks, the cotton plugs in some of the flasks occasionally came in contact with the contents and absorbed small but possibly significant amounts of the culture media. To prevent this, 250 milliliter Delong culture flasks with metal closures were used in the second experiment.

Although chemical conditions were altered for each experiment, the culture media for both experiments consisted of 100 milliliters of well water fortified with nitrogen, phosphorus, trace minerals and vitamins. A chemical analysis of the well water revealed the following chemical composition: chlorides-10.0 mg/l; sulfate-39.0 mg/l; iron-0.04 mg/l; calcium hardness as $CaCO_3$-20.0 mg/l; total alkalinity as $CaCO_3$-332.0 mg/l; total phosphate-0.03 mg/l; orthophosphate-0.01 mg/l; nitrate-2.3 mg/l; and pH-8.5. Nitrogen was added in the form of sodium nitrate ($NaNO_3$) and phosphorus was added as potassium phosphate (K_2HPO_4). Potassium phosphate also served as the carrier for the radioactive phosphorus. Various trace minerals and vitamins were supplied to the media by use of a commercial vitamin

in dilute proportions.

The first experiment was designed to substantiate the Watt-Hayes Model under constant laboratory conditions and to calculate phosphorus exchange rates from the generated data. To begin with, .4 mg. of nitrogen as nitrate and .1 mg. of phosphorus as phosphate were added to each of ten culture flasks. If the amounts of nitrogen and phosphorus in the water are considered, the N/P ratio was 4.1/1. To each flask, 2 milliliters of an algal culture consisting principally of Chlorella, but also including Ankistrodesmus, Scenedesmus, Oscillatoria, Phormidium and Euglena, was added. The percent transmittance of the parent culture was 31.5. It should be noted that the algae were taken from a nearby oxidation pond, and in the same water, cultured in the laboratory for three days prior to inoculation. Therefore, the culture was not bacteria free. Since there is a definite symbiotic relationship between the algae and bacteria in a natural environment, it was important in this study that they both be present and in somewhat normal proportions. Each flask was then inoculated with a constant amount of radioactive phosphorus ($K_2H^{32}PO_4$) and analyzed daily for three weeks.

The second experiment, if results from the first experiment were favorable, was designed to subject the Watt-Hayes Model to varying conditions, and from the

resultant data, disclose existing relationships which might be predictable. It was conducted under the same physical conditions, but the chemical conditions were quite different. An attempt to hold the pH of the cultures constant was made by adding 1.5 grams of tris buffer to each flask. Later, as the pH in the flasks began to rise, 5 percent CO_2 was bubbled twice daily through each flask. This effectively lowered the pH without other noticeable changes. Twenty flasks separated into four groups of five were employed. In the first group, the N/P ratio was 4/1 (NO_3-N/PO_4-P) with phosphorus concentrations of .1 mg/l, 1 mg/l, 3 mg/l, 6 mg/l and 10 mg/l. The phosphorus concentrations of the second group were the same but the N/P ratio was changed to 8/1. The N/P ratios of the third and fourth groups were 2/1 and 1/1 respectively. Both groups contained phosphorus concentrations ranging, in 5 mg/l increments, from 5 mg/l to 25 mg/l. All concentrations are reported in mg/l N and mg/l P. All the flasks were inoculated with 3 milliliters of a 50 percent transmittance algal culture very similar in composition to that used in the first experiment. Labeled phosphorus ($K_2H^{32}PO_4$) was added to each flask in constant amounts that would not significantly increase the total phosphorus concentration. Flasks 1-10 were analyzed periodically for five weeks. It became apparent after a few days that the cultures containing from 10 to 25 mg/l P.

were not responding in a comparable manner with those cultures with lower phosphorus concentrations. Therefore, on Sept. 30 cultures 15 and 20, both containing 25 mg/l P., were replaced by two cultures having .5 mg/l P. and N/P ratios of 4/1 and 8/1.

During the first experiment, ^{32}P was counted with a deep-well scintillation counter (photomultiplier tube and plastic phosphor for beta counting) with an efficiency of 37 percent and background of 120 $\pm$ 5 c.p.m. A Beckman liquid scintillation system which counted ^{32}P with 70 percent efficiency was used in the second experiment. The fluor solution used consisted of 100 milliliters of a concentrated fluoralloy solution (Beckman TLY) and 30 milliliters of Beckman Bio-Solv diluted to 1 liter with analytical grade toluene.

The relative standard deviation of all the sample counts ranged from 1.2 to 7.6 percent. However, a range of 1.5 to 4 percent encompassed 92 percent of the sample counts.

The same analytical procedures and techniques were employed throughout the experimentation. The PP-32 (particulate phosphorus) was taken as the percent difference between the total ^{32}P in the culture and the ^{32}P remaining after filtration through a .45 μ pore diameter filter. A .1 milliliter sample and a .1 milliliter aliquot of a 2 milliliter filtered sample from each culture were

counted to determine the total amount of ^{32}P, and the amount of ^{32}P in the filtrate. A check of the total amount of ^{32}P was made by calculating the ^{32}P activity from a knowledge of the initial dosage and its decay rate.

The DIP-32 and DOP-32 in the filtrate were separated chromatographically and identified by their respective Rf values. The chromatograms were scanned and recorded with a Baird-Atomic radiochromatogram scanner. The areas under the organic and inorganic phosphorus peaks were then measured with a planimeter. By dividing the total area into the area of either the DIP-32 or DOP-32 fraction, the percentage of organic and inorganic phosphorus compounds was obtained. The chromatograms were prepared by placing 100 µl samples of the filtrate on Whatman #1 paper which had been previously washed in 0.1 N HCl and distilled water. They were then developed in methanol, formic acid, and water, (80:15:5), using unidimensional, descending chromatography. Of the many solvents tested this was selected as the best for a rapid separation of the phosphoric esters.

The phosphate compounds formed in the algal mass (PP-32) were also analyzed chromatographically. For each run, several samples were pooled, centrifuged and the supernatant decanted. The phosphorus compounds were then extracted from the algal mass by the addition of boiling absolute ethanol, followed by boiling 80 percent ethanol.

This mixture was taken down to dryness using a Flash Evaporator and then taken up in 2 milliliters of water. Samples were placed on chromatographic paper and developed in the same manner as the filtrate. The chromatograms were scanned and the percent of each compound determined.

Prior to working with the batch cultures of algae, standards of several phosphoric esters were freshly prepared and chromatograms (paper, unidimensional, ascending and descending; two-dimensional paper; and thin-layer, uni- and two dimensional) were developed in various solvents (Table I) to determine which was best suited for this study. Methanol, formic acid and water (80:15:5) was selected and using this solvent, the Rf values for the phosphoric esters were determined (Table II).

Development and detection of the phosphate compounds was accomplished by spraying with the following solution: 5 milliliters of 60 percent perchloric acid, weight by weight, 25 milliliters of 4 percent ammonium molybdate ($(NH_4)_2MoO_4$), weight by volume; and 10 milliliters of 1N HCl; all incorporated and made up to a total volume of 100 milliliters. The sprayed papers were then treated by heating in an oven at 85°C for one minute and then illuminated with ultraviolet light for 10 minutes at a distance of 10 centimeters. A GE germicidal lamp rated at 25 microwatts of 2537 Å radiation per sq. cm. at one meter was used. All of the

TABLE I

SOLVENTS TESTED FOR CHROMATOGRAPHIC ANALYSIS

Solvent	Proportion
Methanol:Formic Acid:Water	80:15:5
Methanol:Acetic Acid:Water	80:15:5
Methanol:Ammonium Hydroxide:Water	60:10:30
nPropanol:Acetic Acid:Water	40:10:50
Butanol:Acetic Acid:Water	74:19:50
Acetone:35% Trichloracetic Acid	60:40

TABLE II

Rf DETERMINATIONS USING METHANOL:FORMIC ACID:WATER (80:15:5)

Compound	Rf	Color Response
Orthophosphate	.70	Yellow-green
3 Phosphoglyceric acid	.55	Blue
Glucose 1-phosphate	.28	Blue-green
Glucose 6-phosphate	.30	Blue-green
Fructose 6-phosphate	.38	Blue
Fructose 1,6-diphosphate	.40	Blue
Adenosine triphosphate	.12	Blue

organic phosphate compounds appeared blue, and the inorganic phosphorus compounds were yellow-green.

Exchange rates were calculated by using the steady state and rate of change equations listed in Appendix A. A sample calculation is presented in Appendix B. The turnover times are the reciprocals of the exchange rates, and by multiplying the amount of phosphorus in a phase by the corresponding exchange rate, the amount of phosphorus transferred per day in each direction can be obtained.

The various relationships between phosphorus concentrations, cell concentrations and exchange rates were developed by multiple linear regression analysis. The expression is of the form

$$\hat{Y} = B_o + B_1 X_1 + B_2 X_2 \ldots + B_n X_n$$

where $\hat{Y}$ is the estimator of the true Y or the dependent variable. B_o represents a constant, B_1 and B_2 are partial coefficients of correlation, and X's represent independent variables.

EXPERIMENTAL RESULTS AND DISCUSSION

Many investigators have attempted to determine the effects of phosphorus on algae growth. In several instances, the attempts failed because the data were inconsistent, irrelevant or non-reproducible. More times than not, the failures could probably be traced to the inherent variability of biological systems. Further complications generally occur when mixed, symbiotic systems are studied. If applicable data are to be obtained, some flexibility in the experimental design is essential, and exact relationships should not be anticipated.

To insure that algal growth was somewhat phosphorus dependent under the conditions of the first experiment (N/P = 4/1; P = 1 mg/l), a preliminary study was conducted. Two flasks with contents identical to those used in the first experiment were inoculated with $KH_2{}^{32}PO_4$ and algae. Growth, expressed as percent transmittance, and phosphorus uptake were monitored for sixteen days. The average values plotted against time are shown in Figures 7 and 8. The two curves coincide closely indicating that growth occurred at approximately the same rate as phosphorus was taken up.

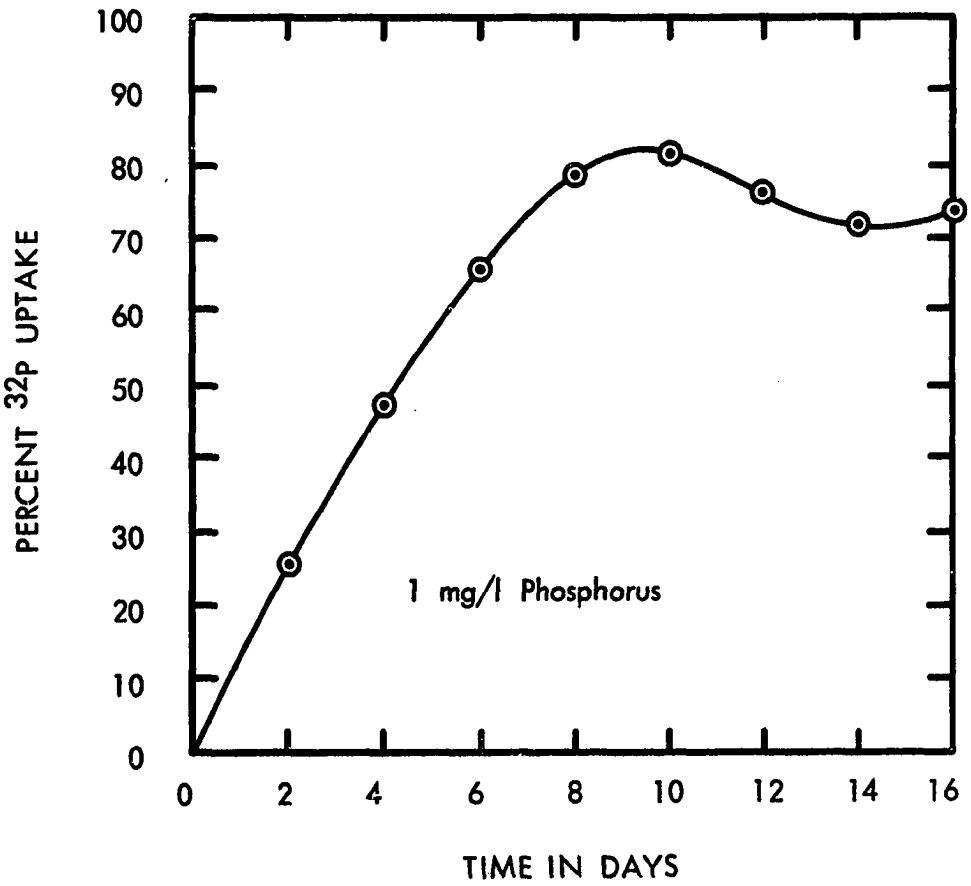


Figure 7. Percent ^{32}P uptake versus time.

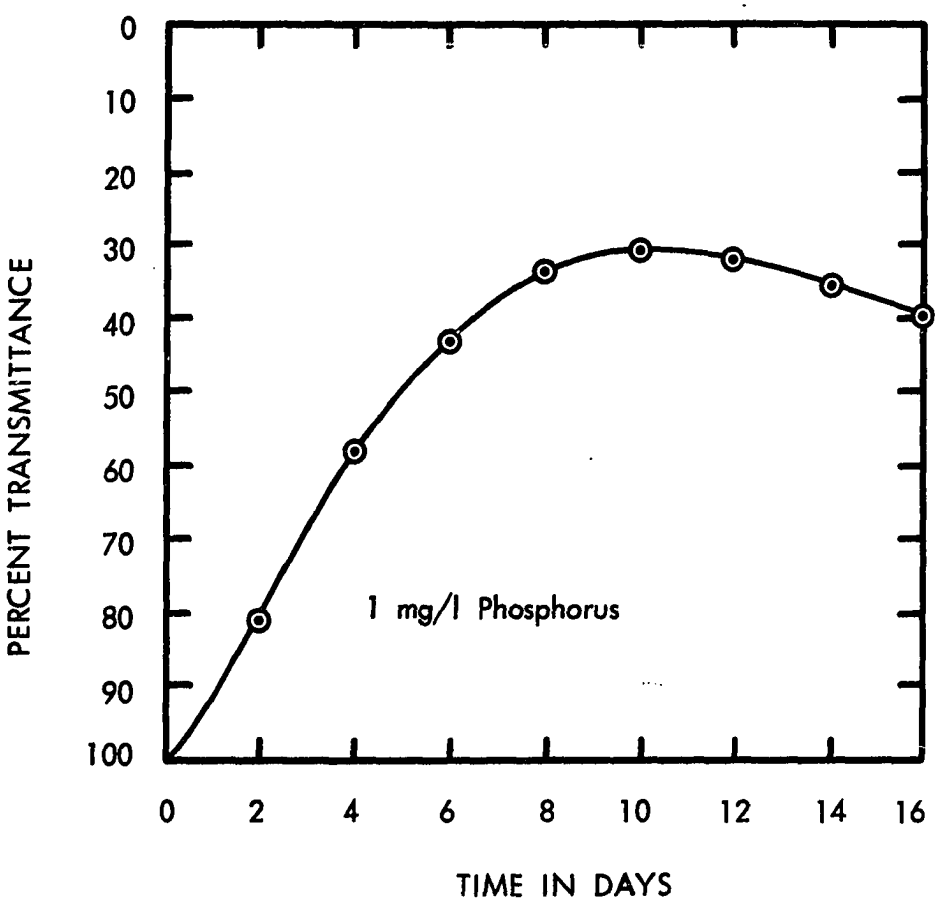


Figure 8. Growth expressed as percent transmittance versus time.

For the first experiment, Figures 9-17 (one of the cultures was spilled) exemplify the transport and exchange of phosphorus in the cultures. As the DIP decreased exponentially, the PP increased exponentially until about the ninth or tenth day when a steady state was established. In cultures numbered 3, 5, 6 and 7, a decrease in the PP concentration was noted about the sixteenth day. The DOP curve normally peaked around the fourth day, declined slightly, leveled off, and in numbers 3, 5, 6 and 7, increased slightly around the sixteenth day. The initial increase in the DOP concentration probably resulted from the release of organic phosphorus compounds from dying bacterial cells. Reid and Gearheart (48) observed a bacterial increase on the second day after inoculation and by the fifth day the bacterial population had significantly subsided. Although no bacterial counts are available to substantiate similar happenings in our cultures, a bacterial decline is a distinct possibility, and can explain the DOP increase. The second increase, which occurred around the sixteenth day, was probably due to the death of an appreciable number of algal cells as indicated by the increased percent transmittance and the color change (green to yellow-brown) in the flasks. It may be surmised that the decrease in PP and increase in DOP on the sixteenth day represents the instability of the steady state condition. It may also

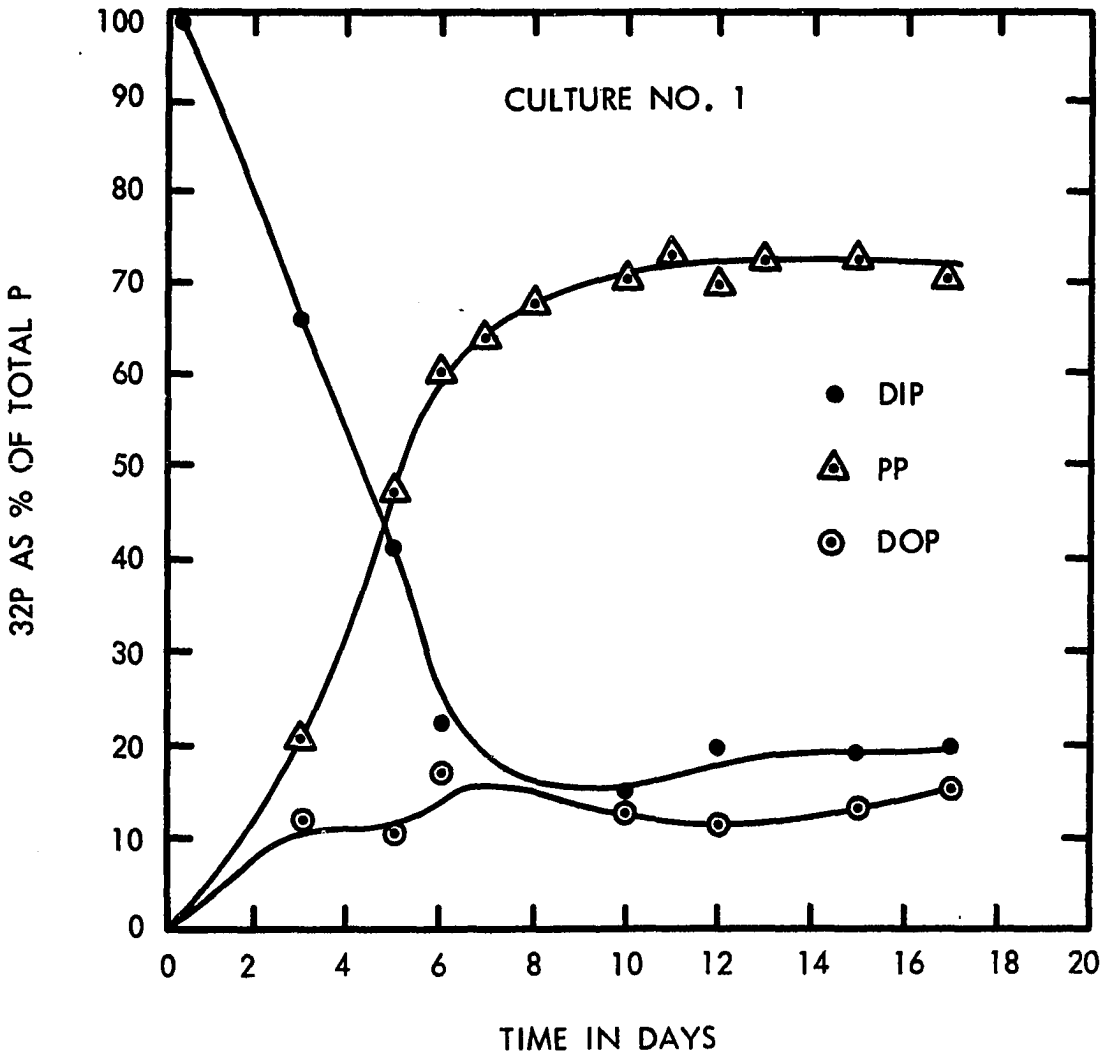


Figure 9. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

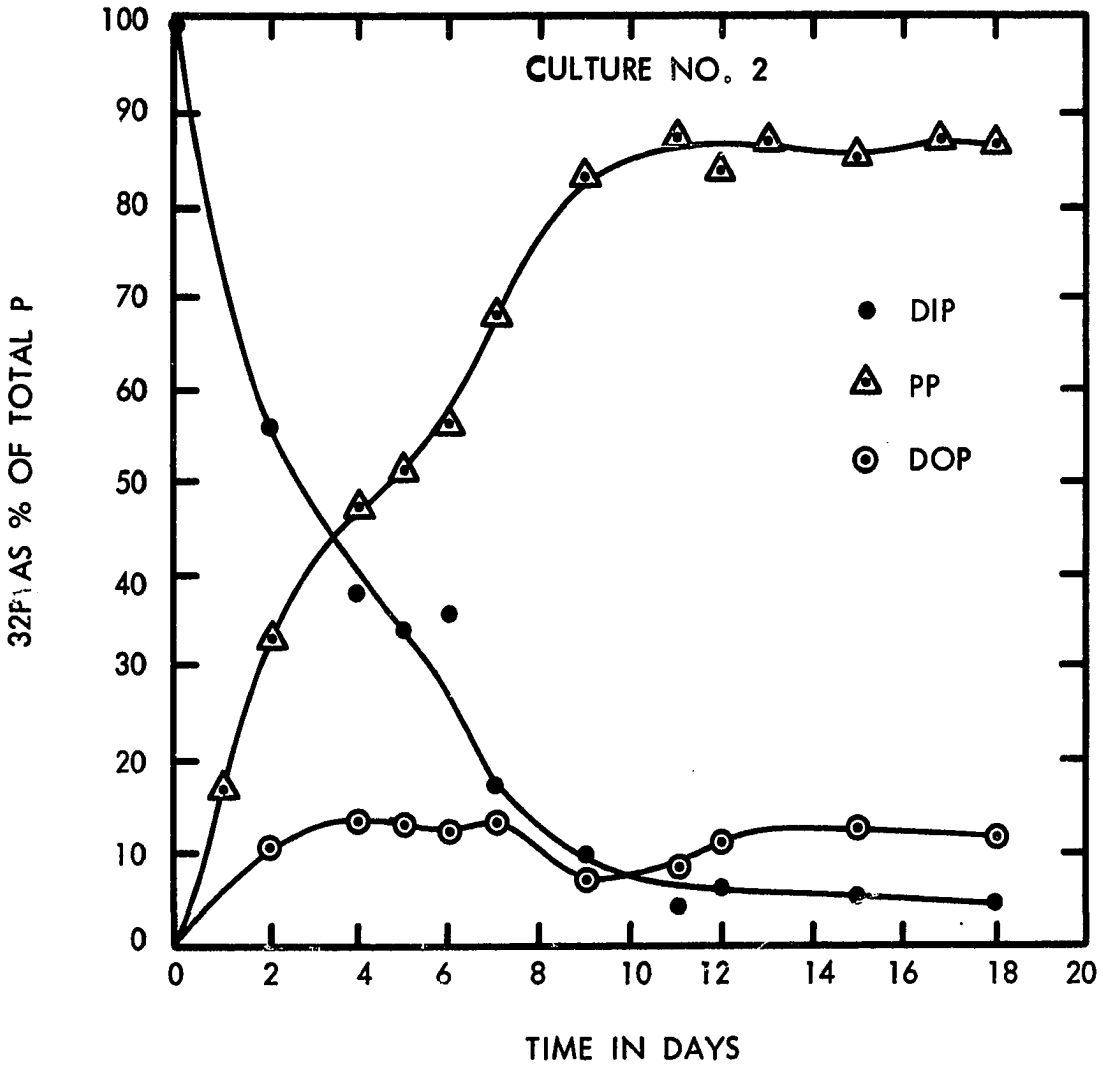


Figure 10. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

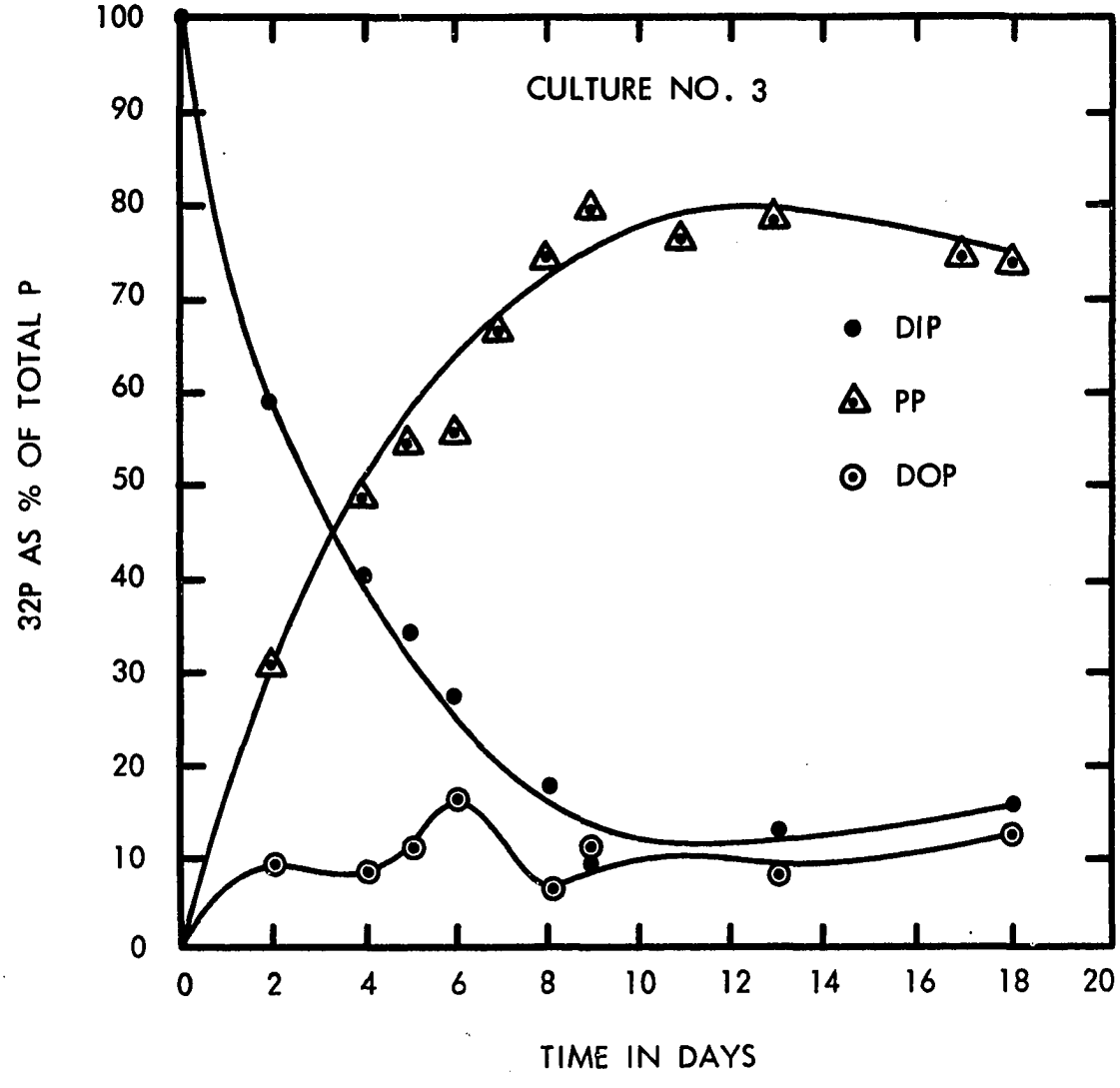


Figure 11. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

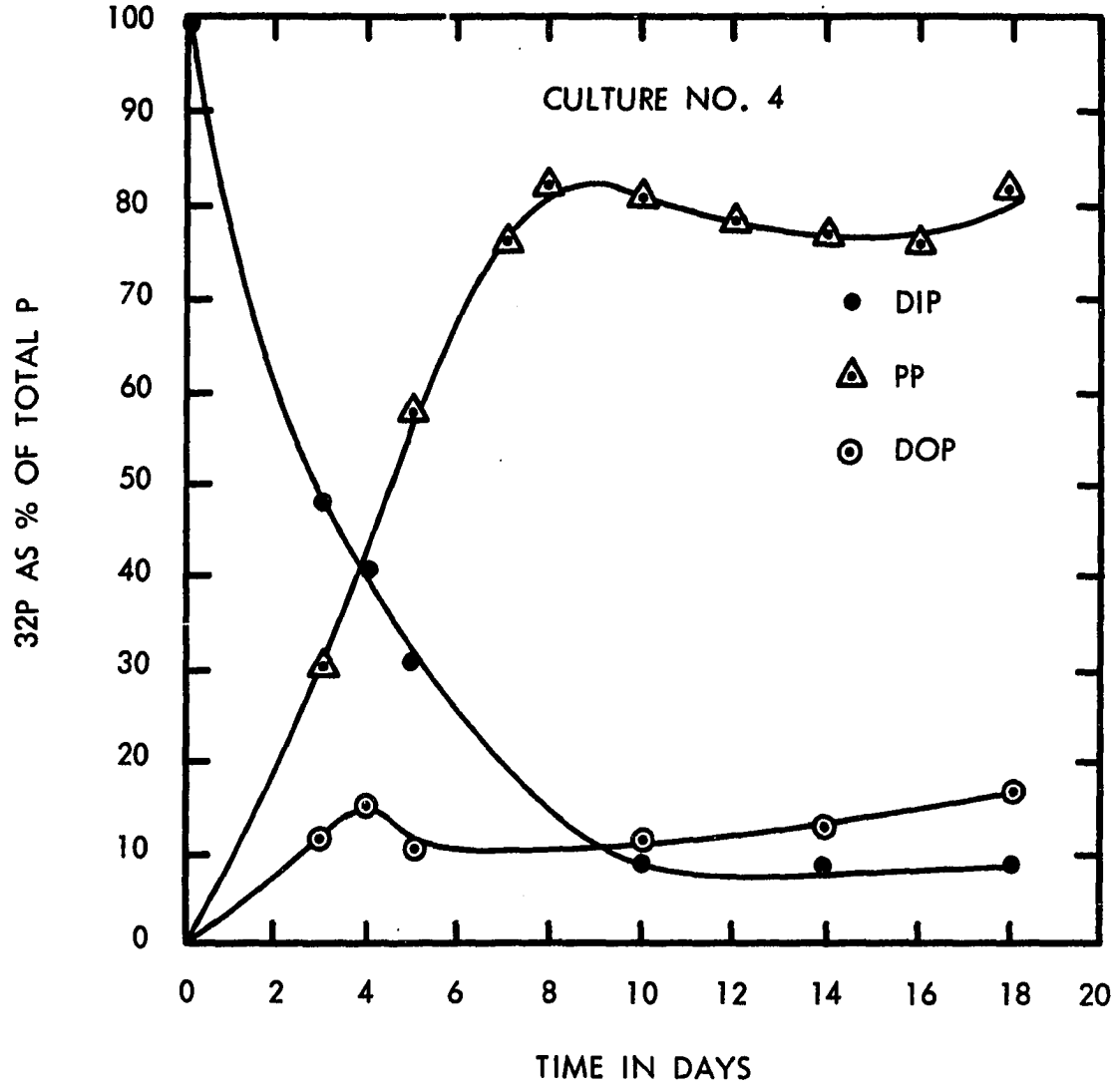


Figure 12. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

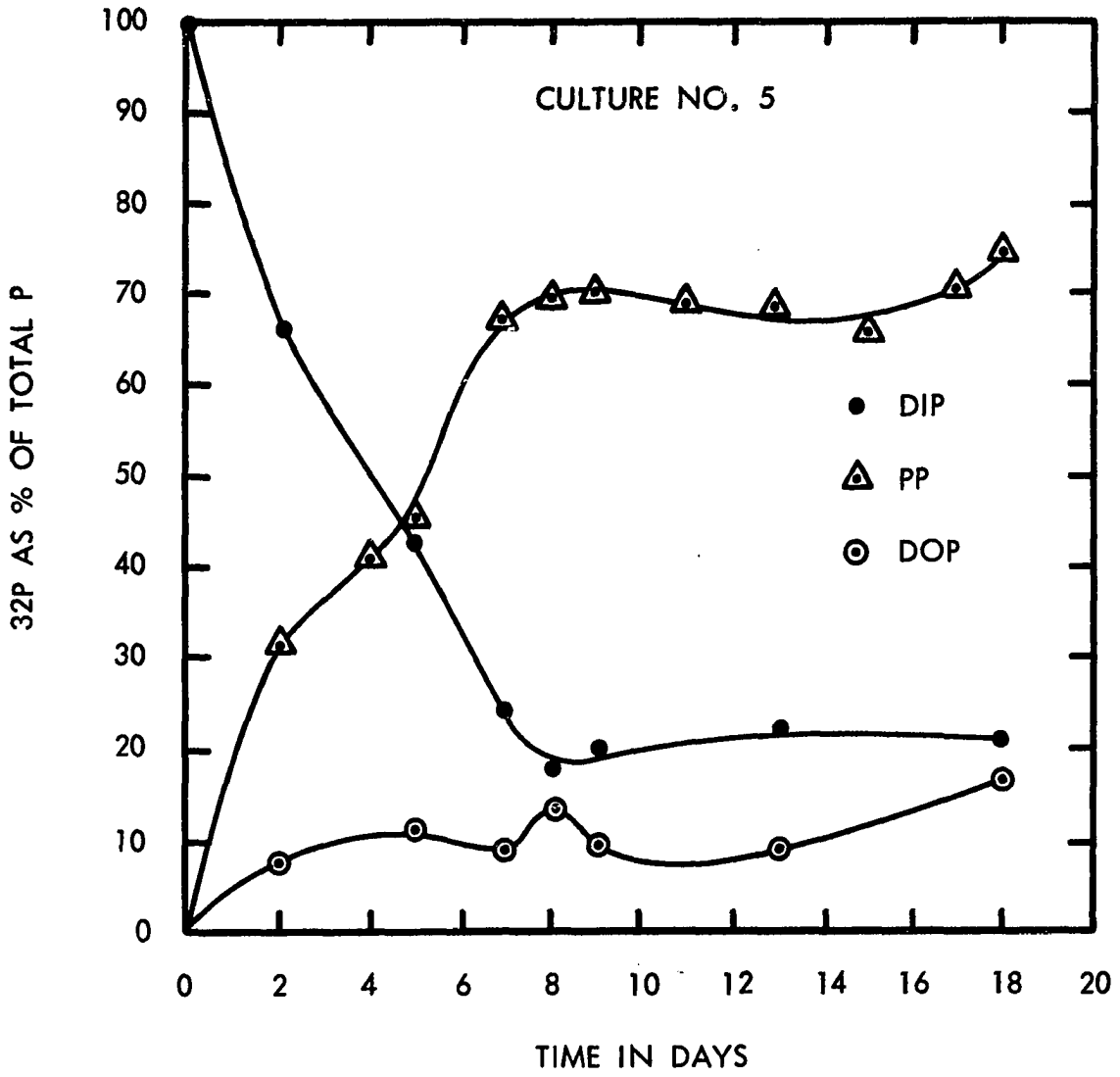


Figure 13. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

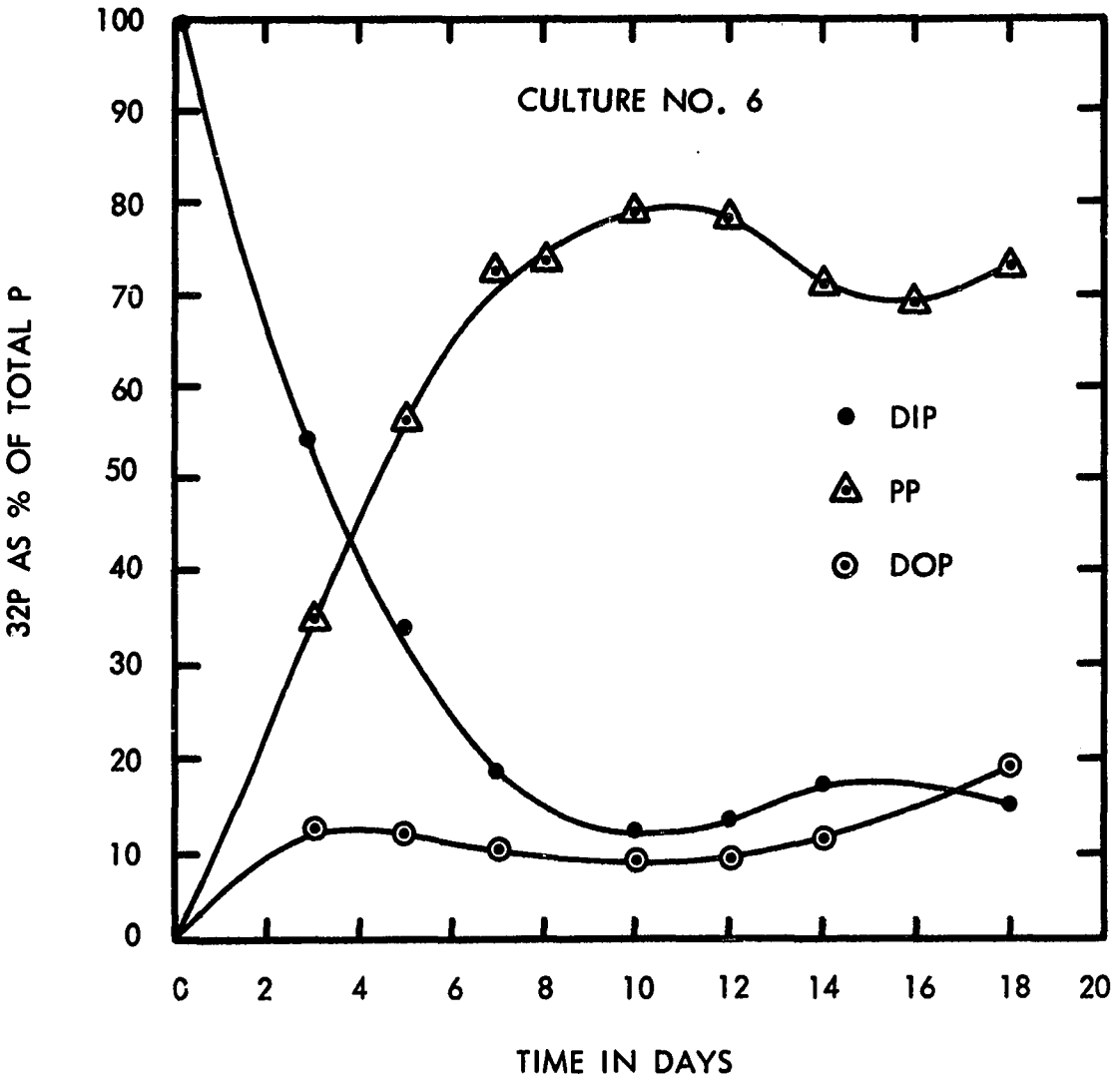


Figure 14. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

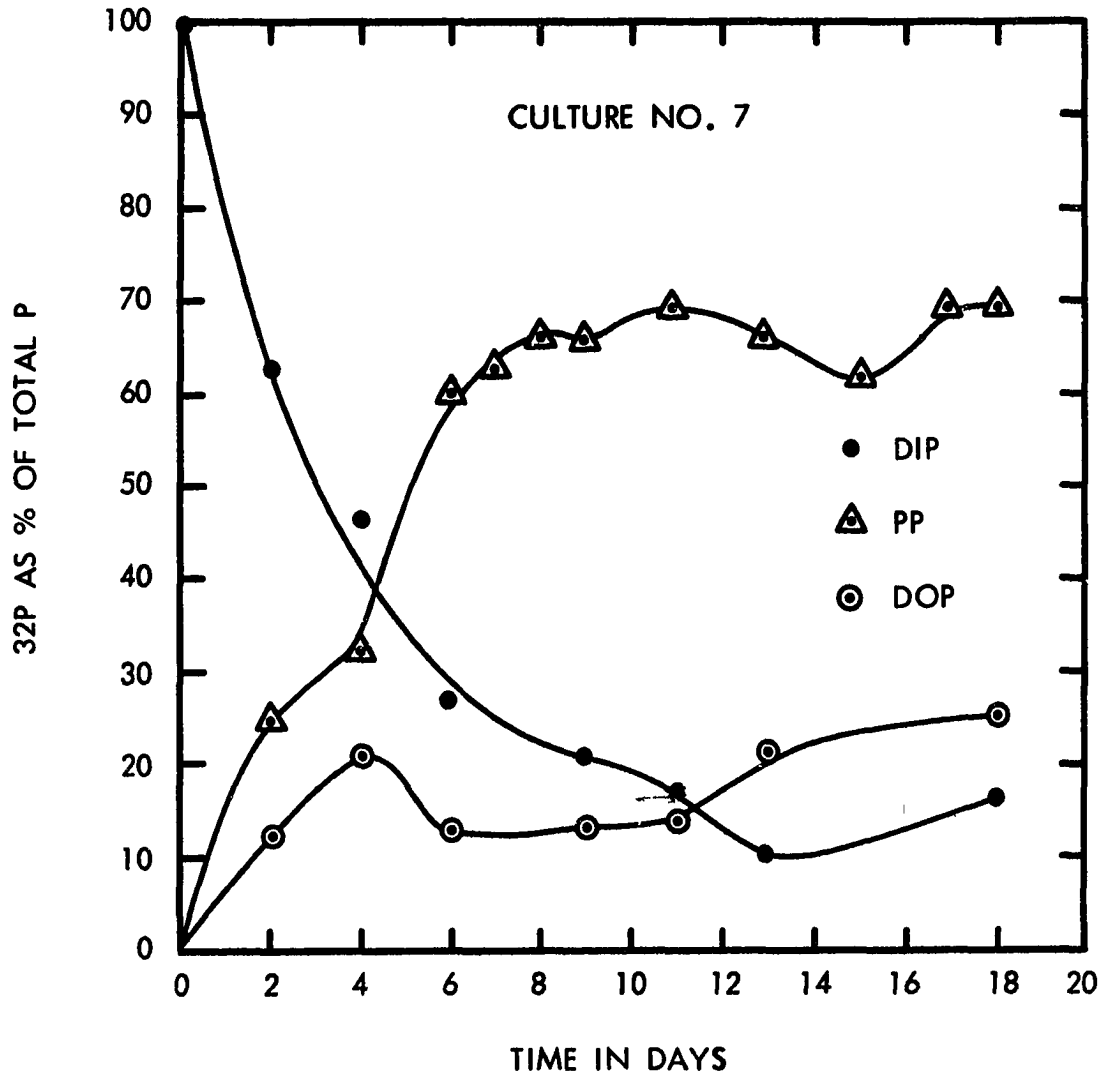


Figure 15. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

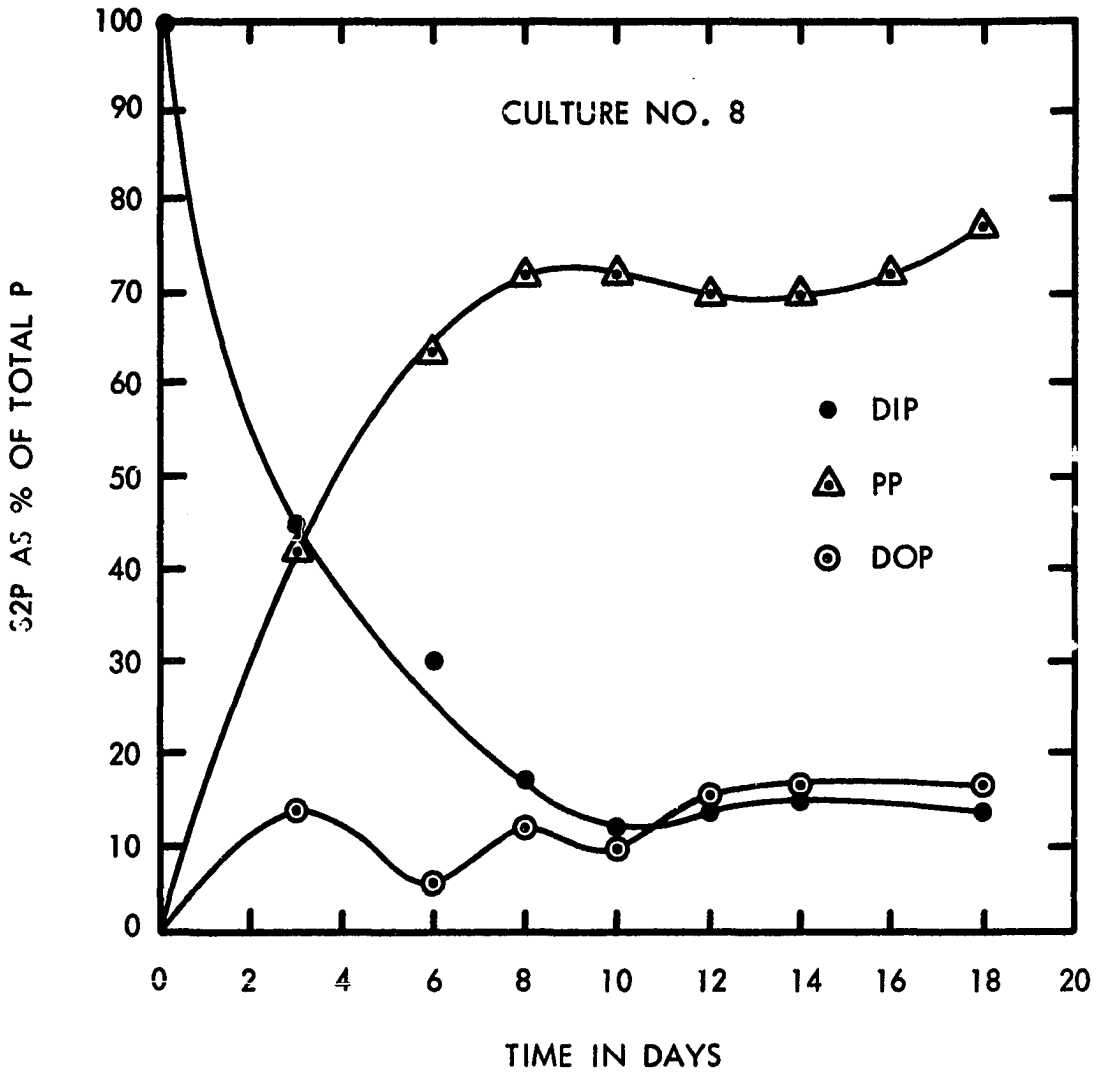


Figure 16. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

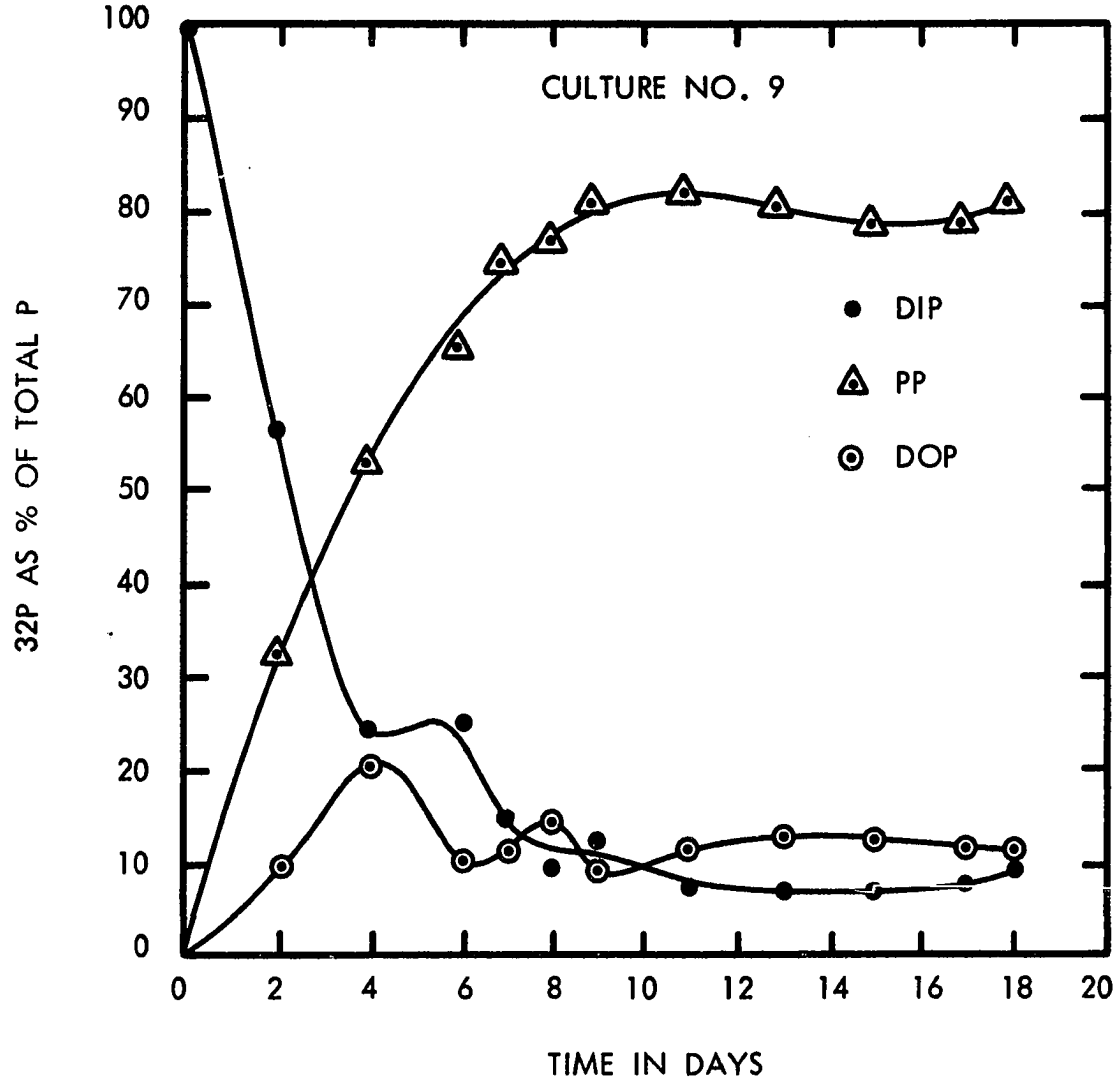


Figure 17. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

be surmised that this change in the steady state condition would not have occurred if the phosphorus supply had been increased by contributions from "sinks" such as sediment. Constant phosphorus additions from such sources might have kept the system in balance. The average minimum transmittance of the nine cultures was 43 percent (175 mg/100 ml of dry algae). Minimum transmittances in all cultures occurred between the ninth and fourteenth days.

The percentage of phosphorus in each of the phases at steady state was determined for each culture by averaging the measured percentages of phosphorus as DIP, PP and DOP immediately following steady state establishment. These values along with the mean steady state phosphorus percentage in each phase are given in Table III.

Using the steady state values from Table III and the equations from Appendix A, phosphorus exchange rates were calculated for each flask (Table IV). The turnover times which are the reciprocals of the exchange rates are also shown in Table IV. In this study, turnover time is defined as the time necessary for an amount of phosphorus equivalent to the total amount in a phase to leave that phase.

Multiplying the average exchange rates from Table IV by the appropriate value for total phosphorus in a phase (mean steady state percentage x .1 mg.) gives one the amount

TABLE III

STEADY STATE PHOSPHORUS CONCENTRATIONS

Culture No.	DIP	PP	DOP
1	8	81	11
2	20	65	15
3	15	74	11
4	8	78	14
5	22	67	11
6	15	74	11
7	17	64	19
8	15	72	13
9	9	79	12
mean $\pm \sigma$	14 ± 5	73 ± 6	13 ± 3

TABLE IV

PHOSPHORUS EXCHANGE RATES AND TURNOVER TIMES

Culture No.	Exchange Rates ($Days^{-1}$)			
	a	b_1	b_2	c
1	.23	.02	.11	.82
2	.19	.06	.14	.61
3	.19	.04	.10	.67
4	.18	.02	.14	.79
5	.19	.06	.14	.85
6	.19	.04	.11	.74
7	.17	.05	.11	.54
8	.19	.04	.10	.55
9	.18	.02	.08	.53
mean $\pm$ σ	$.19 \pm .02$	$.04 \pm .02$	$.12 \pm .02$	$.68 \pm .12$

Turnover Times (Days)

DIP-32 = $1/a = 5.26$

PP-32 = $1/(b_1 + b_2) = 6.25$

DOP-32 = $1/c = 1.47$

of phosphorus being exchanged per day in each flask (Figure 18).

Values obtained for each of the exchange rates and steady state concentrations from each flask were relatively uniform. Because of this, the assumption that predictable steady state concentrations and exchange rates result from a given initial phosphorus concentration was investigated in the second experiment.

During the first experiment the filtrate from the cultures was analyzed chromatographically to determine the percentages of inorganic and organic phosphorus. It can be seen from the scanned chromatogram print-outs that over a period of time there was an increase in the amount of DOP and a decrease in the amount of DIP in the filtrate (Figure 19). One must keep in mind, however, that as DIP decreased and DOP increased, total activity decreased which reduced the peak heights.

The DOP phase was resolved into six organic compounds which are the same as those presented in Table II. No attempt was made to determine the amount of each organic compound because the exchange rates representing DOP were for the phase as a whole.

Algal extracts were analyzed chromatographically on two occasions. The same six organic compounds were identified from the extracts and, in addition, significant amounts

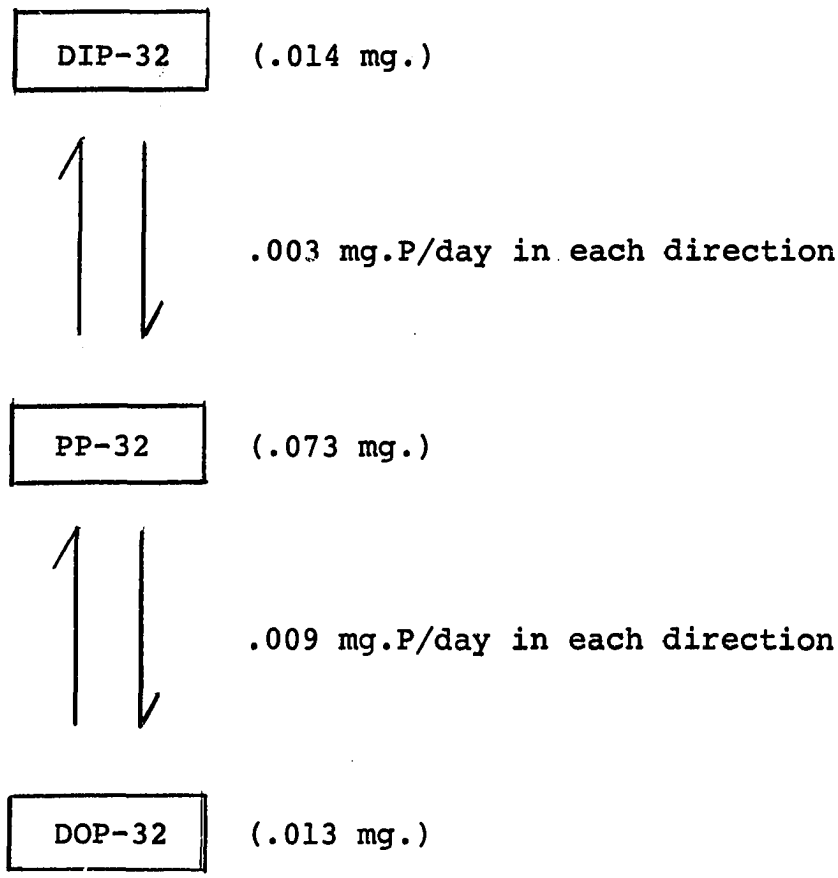


Figure 18. Phosphorus exchange values for batch cultures of algae containing 1 mg. of phosphorus.

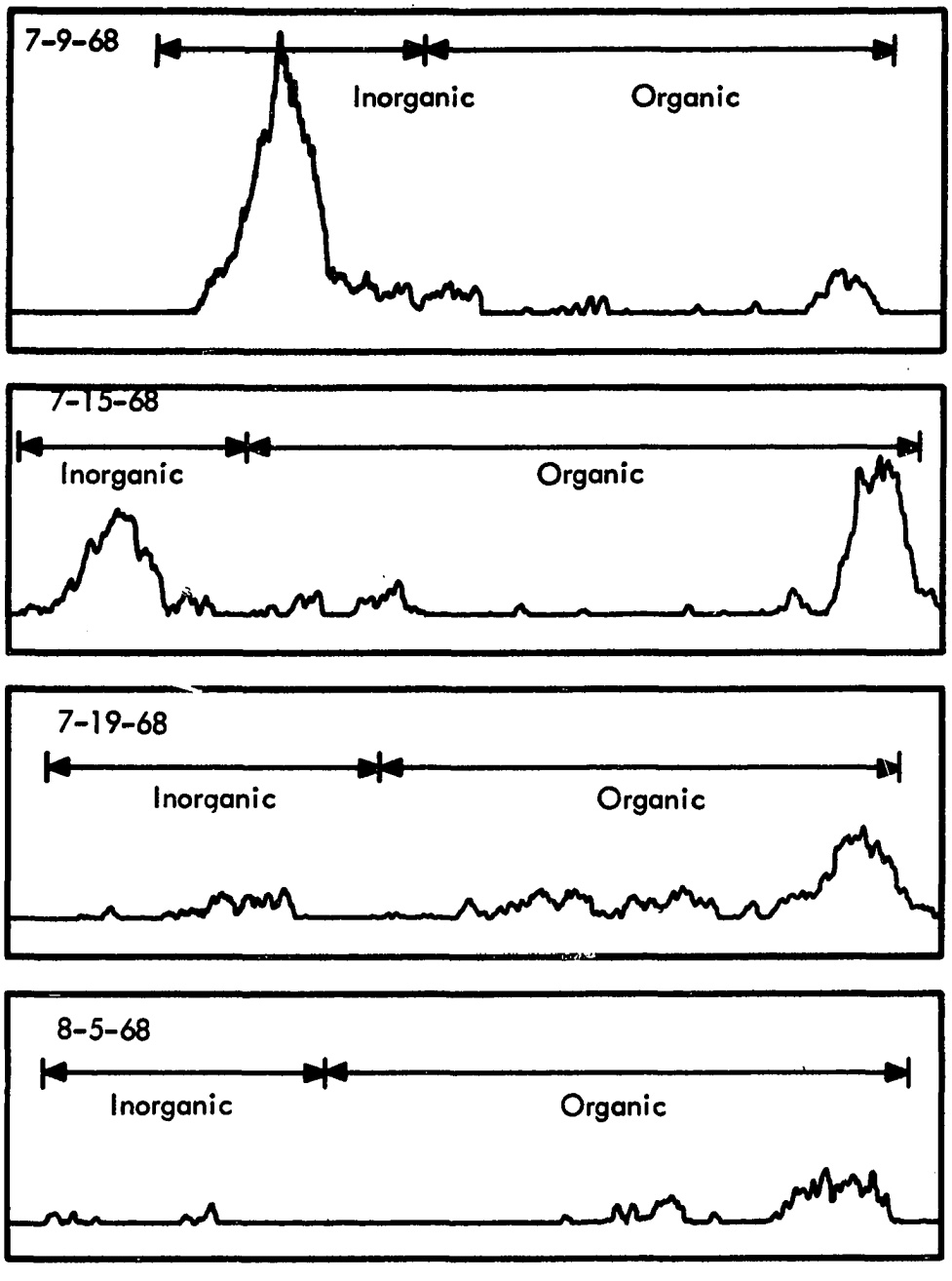


Figure 19. Chromatogram scan showing the eventual conversion of DIP into DOP in the filtrate.

of inorganic phosphorus were detected. The presence of inorganic phosphorus could be explained in two ways; either inorganic phosphorus was stored in the cells or the extraction procedure caused some of the organic phosphorus compounds to be hydrolyzed.

In the second experiment the results of an extractive and enzymatic analysis described by Fitzgerald and Nelson (86) indicated that inorganic phosphorus was indeed stored in algal cells. This analysis was employed to determine if phosphorus was stored at various concentrations. Under existing experimental conditions, phosphorus was not stored until media concentrations greater than 1 mg/l P were reached. Only small amounts of phosphorus were stored by the cells in media containing 3 mg/l P. Above this concentration, the cells contained enough surplus phosphorus to survive for several days in a phosphorus free solution.

At the outset of the second experiment, algal growth did not coincide with phosphorus uptake as it did in the first experiment. This is shown by comparing Figures 20 and 21. All of the cultures exhibited a lag phase until the sixth day when exponential growth began. This lag phase can be attributed to ecological conditions preceeding the experiment. The algae were maintained for a week in the original oxidation pond water before being introduced into the culture media. As a result, the physiological state of the organ-

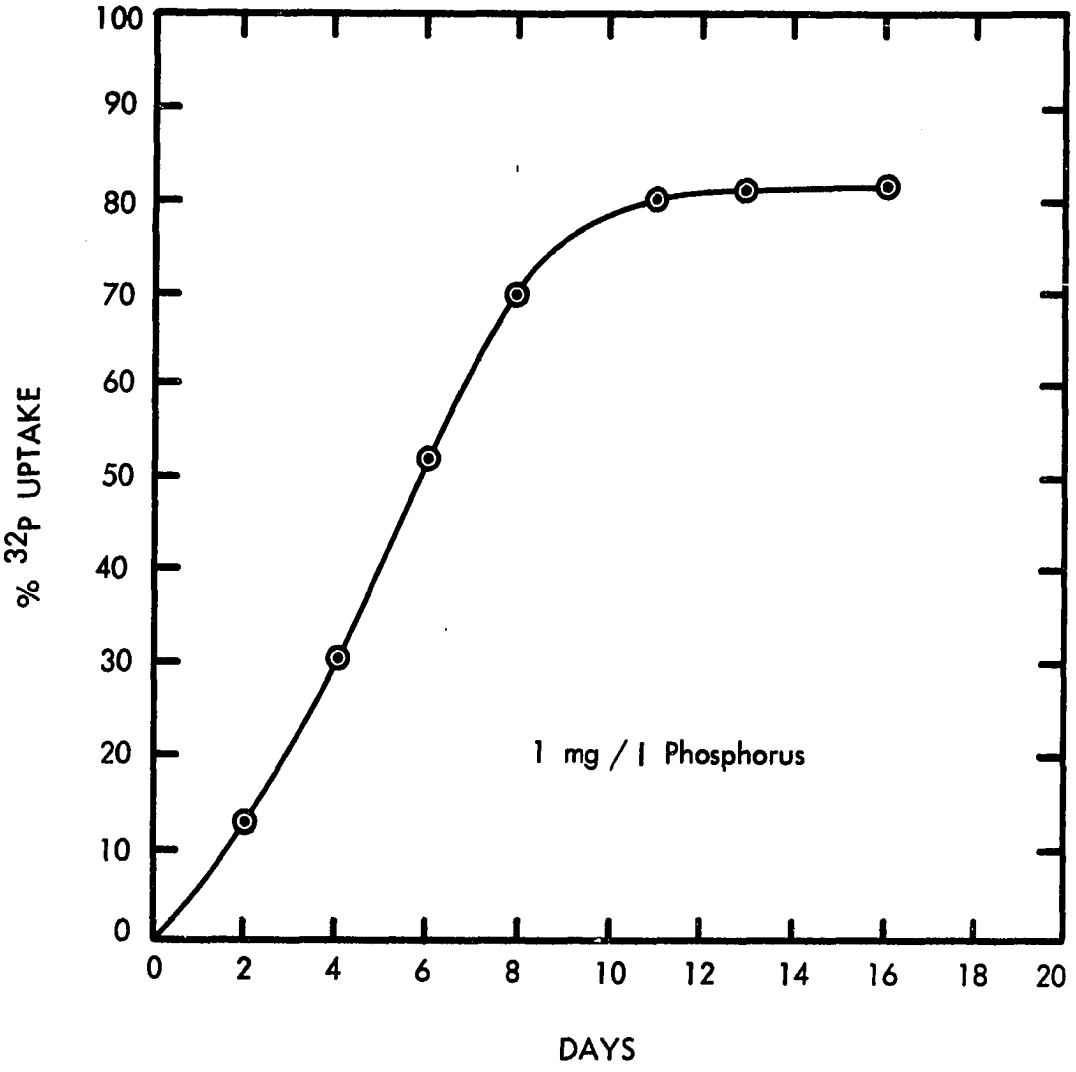


Figure 20. Percent ^{32}P uptake versus time.

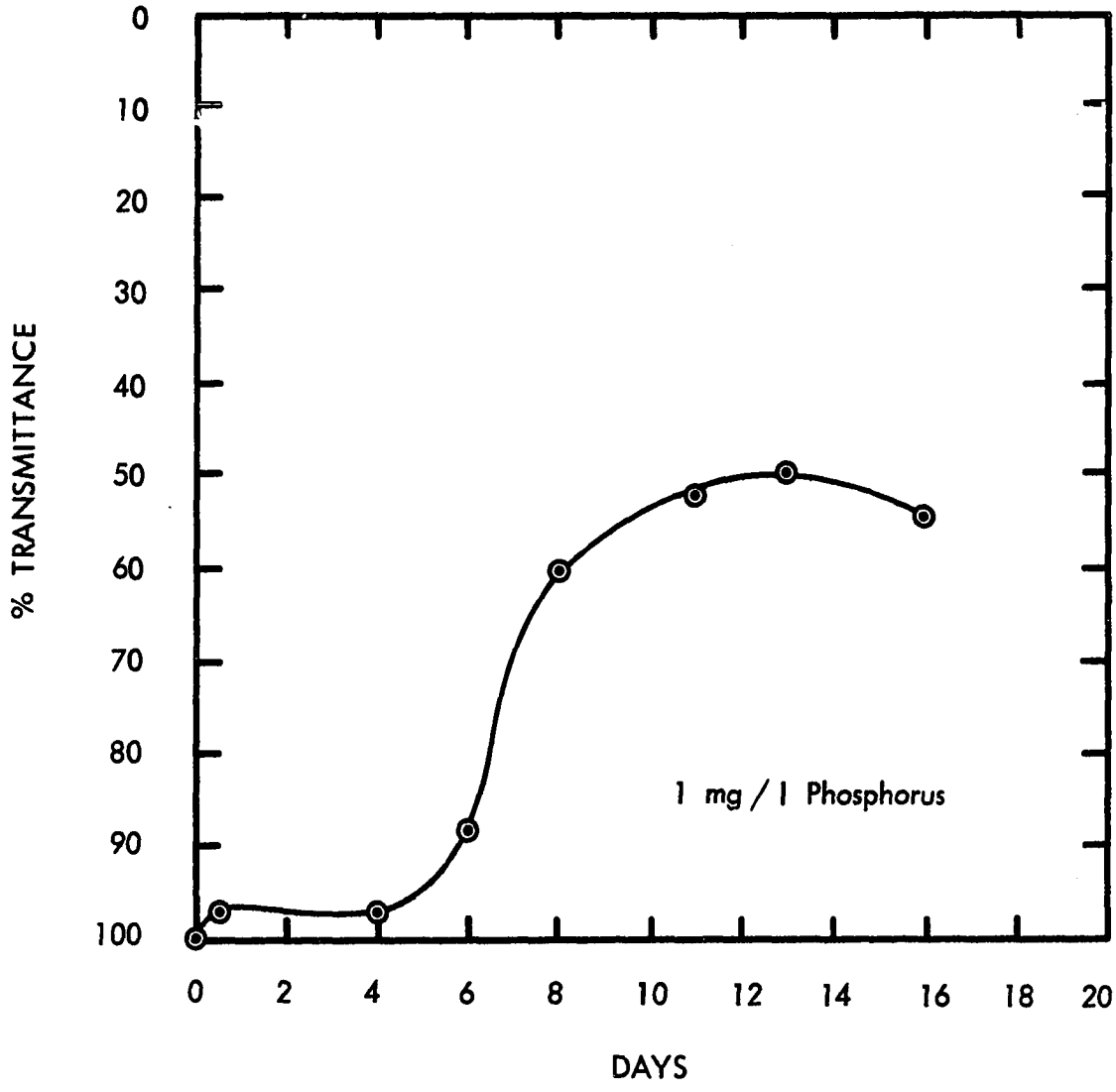


Figure 21. Growth expressed as percent transmittance versus time.

isms was probably poorer than in the preliminary study and the first experiment. After the lag phase, however, algae growth and phosphorus uptake generally correspond. In both instances in which plots were made, the phosphorus concentration was 1 mg/l P and the N/P ratio was 4/1. In each, 80 percent of the phosphorus was taken up by the tenth day, but maximum growth occurred three days later in the second experiment.

The transfer of phosphorus through the DIP, PP and DOP phases is represented graphically in Figures 22-41. A definite steady state condition was established only in those cultures containing 3 mg/l P or less. They behaved in much the same manner as the cultures from the first experiment. In these cultures (no.'s 9, 10, 11, 12, 15, 16, 17 and 18), steady state was established between days 8 and 11. There was an exponential decrease in DIP and a simultaneous exponential increase in PP until steady state was established. The DOP concentration reached a maximum around the sixth day, then tapered off, and in most instances, increased slightly sometime after the sixteenth day.

The remaining cultures, which contained phosphorus concentrations in excess of 3 mg/l P, exhibited no well defined steady state conditions, although approximate steady state values are given for them in Table V. DIP decreased and PP increased over the experimental period, but the

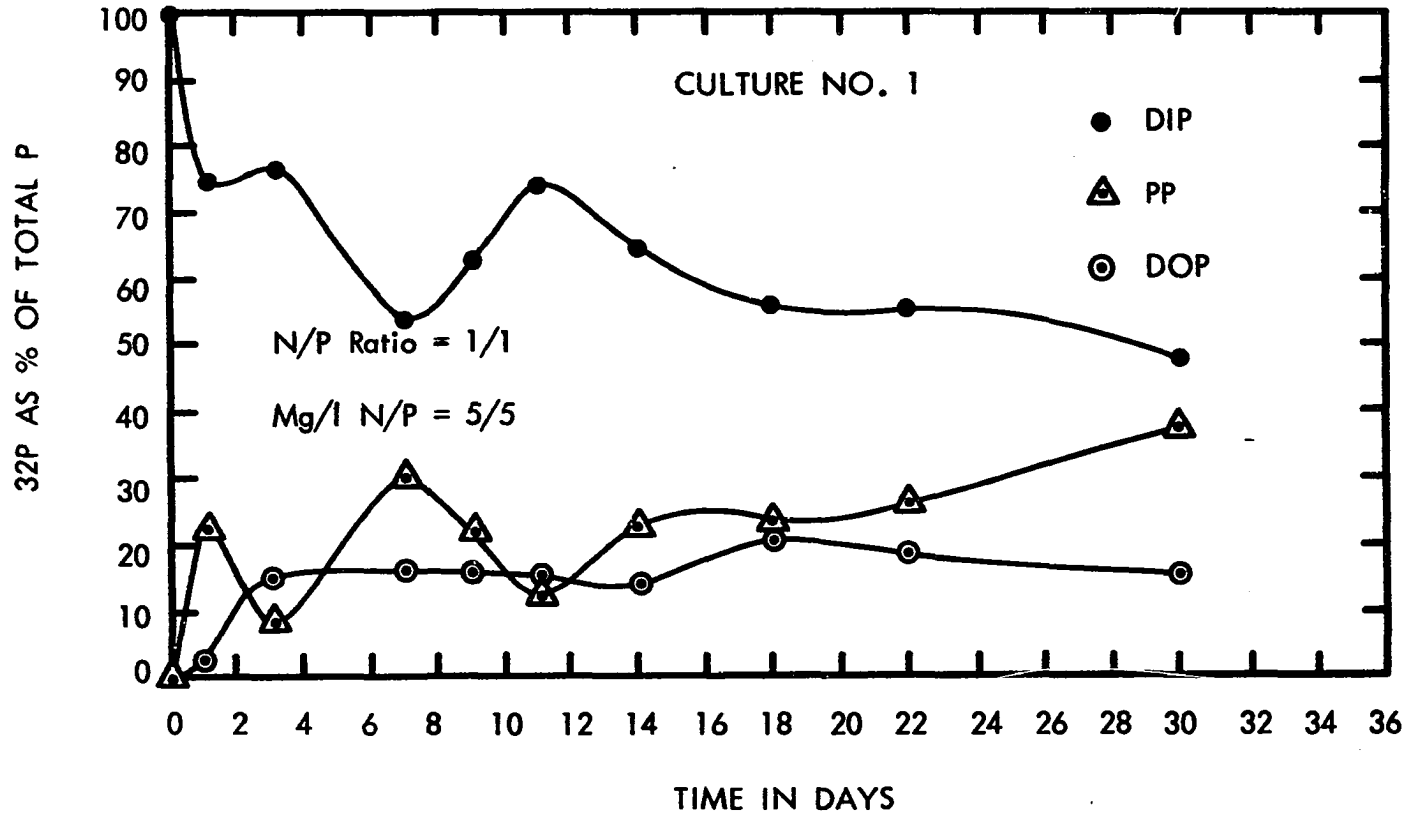


Figure 22. Percentage of 5.0 mg. DIP Incorporated into PP and DOP with time.

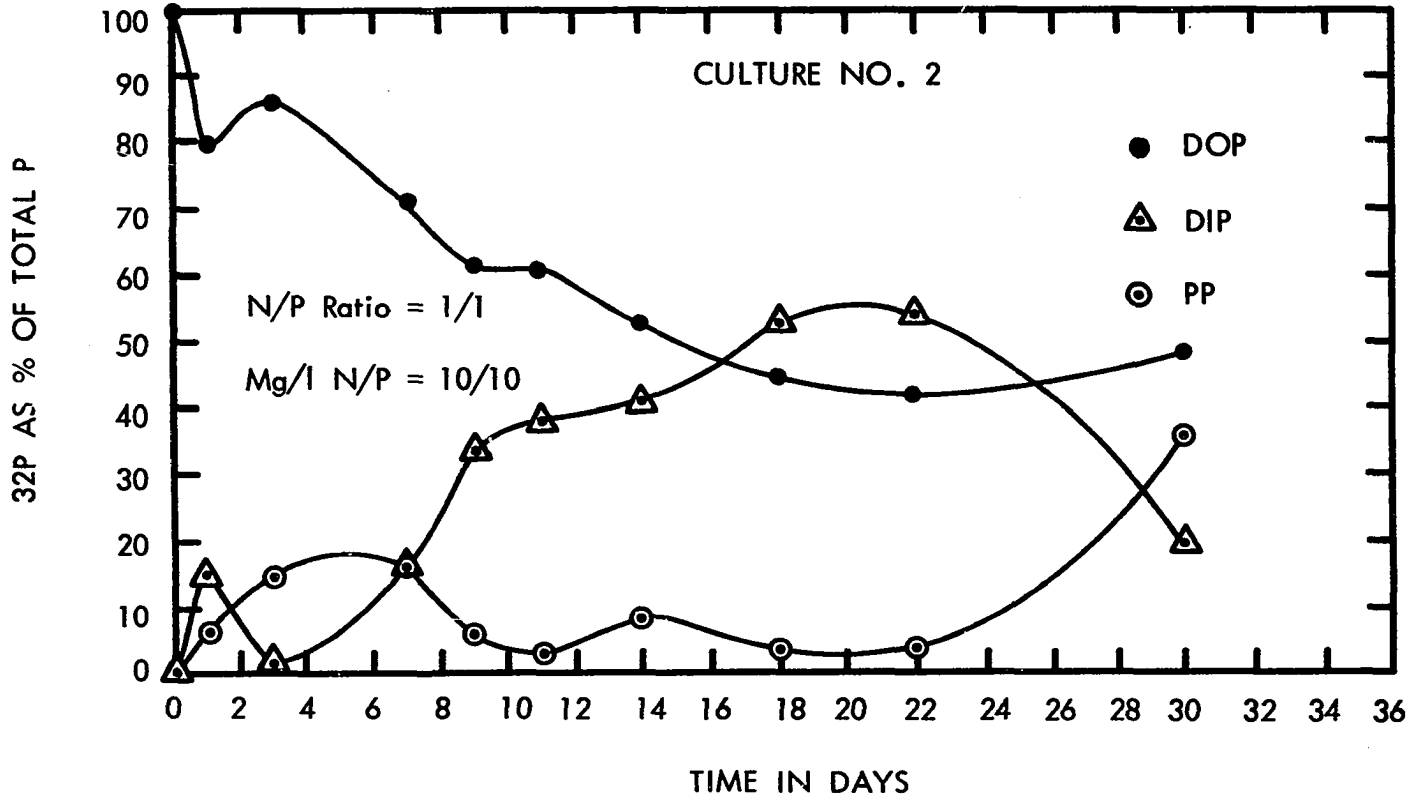


Figure 23. Percentage of 10.0 mg. DIP Incorporated into PP and DOP with time.

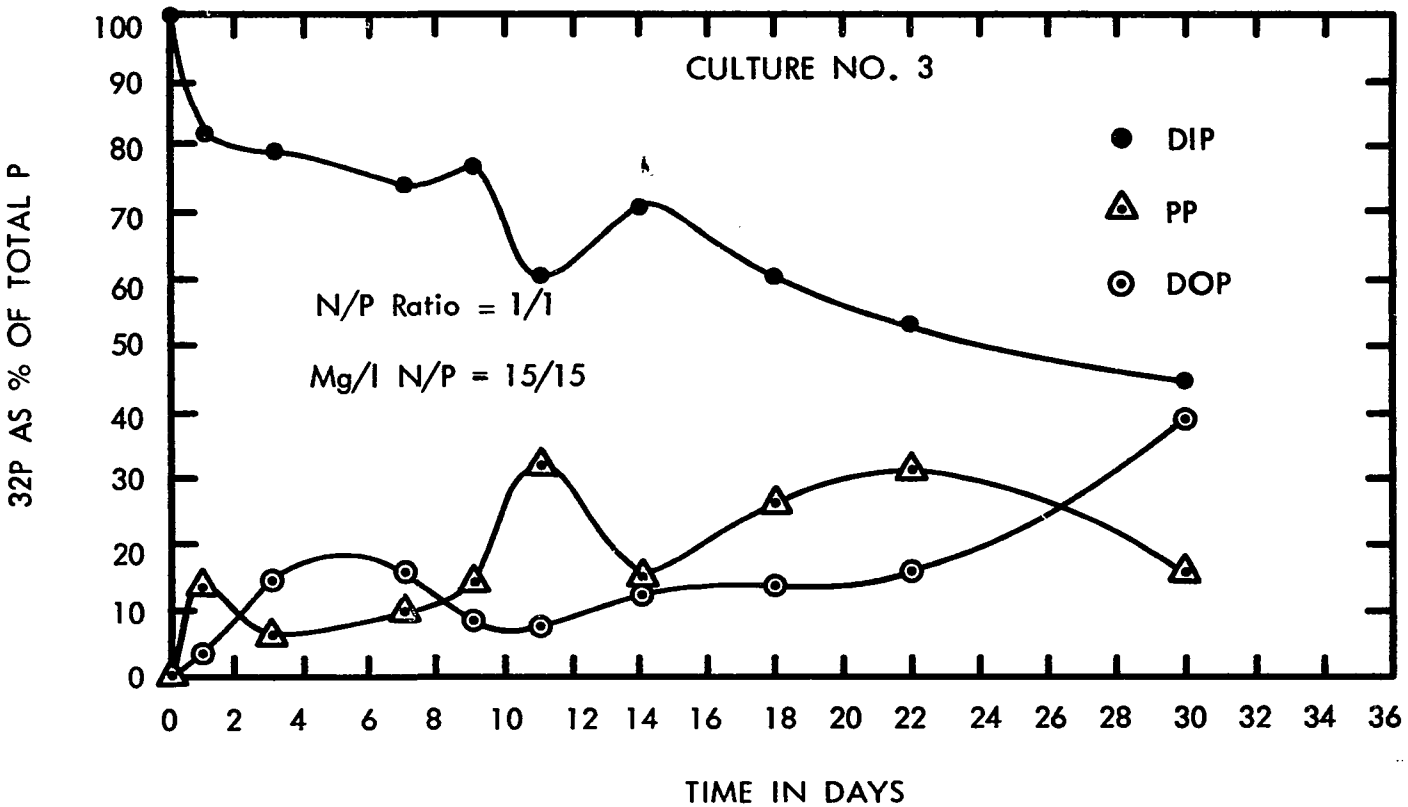


Figure 24. Percentage of 15.0 mg. DIP Incorporated into PP and DOP with time.

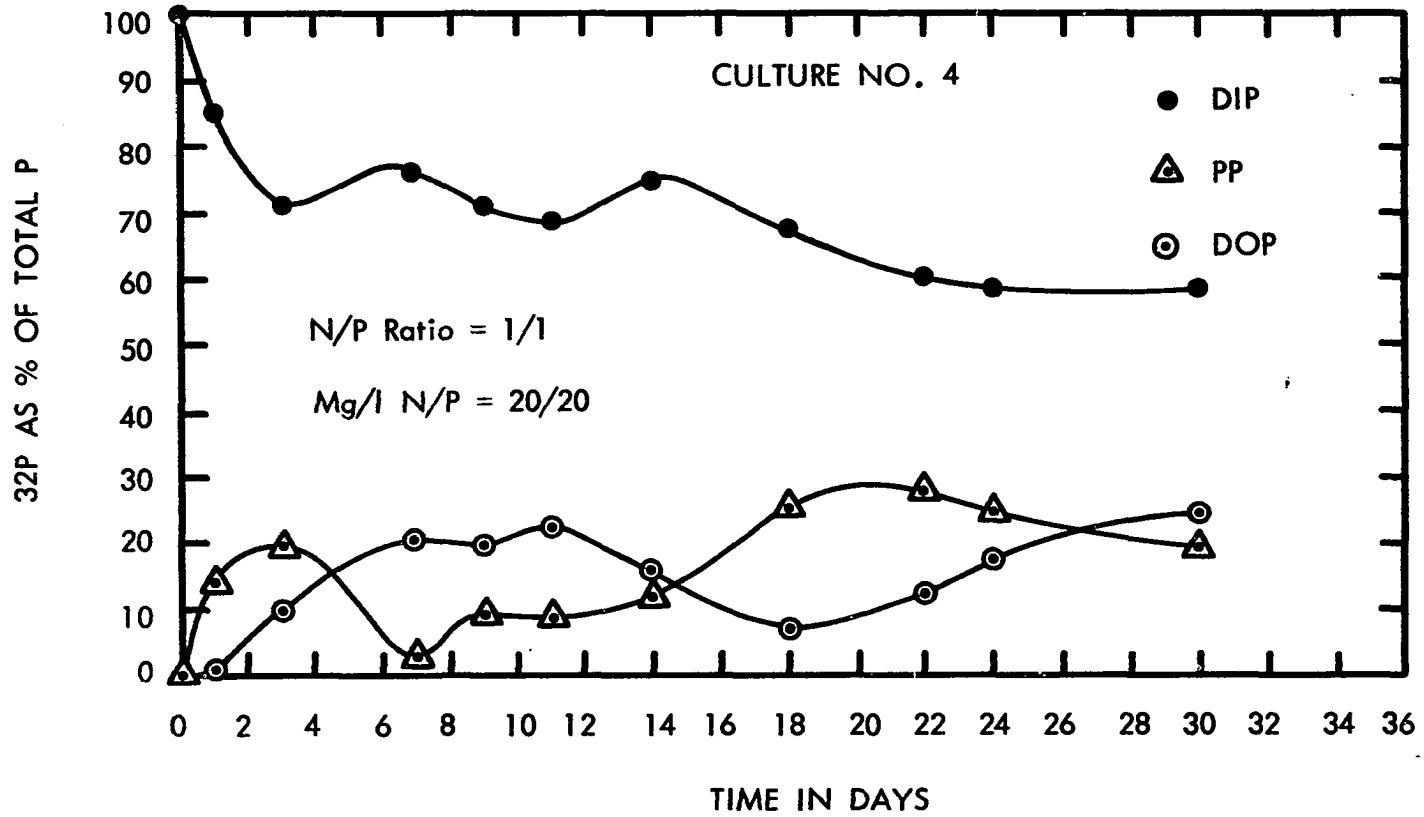


Figure 25. Percentage of 20.0 mg. DIP Incorporated into PP and DOP with time.

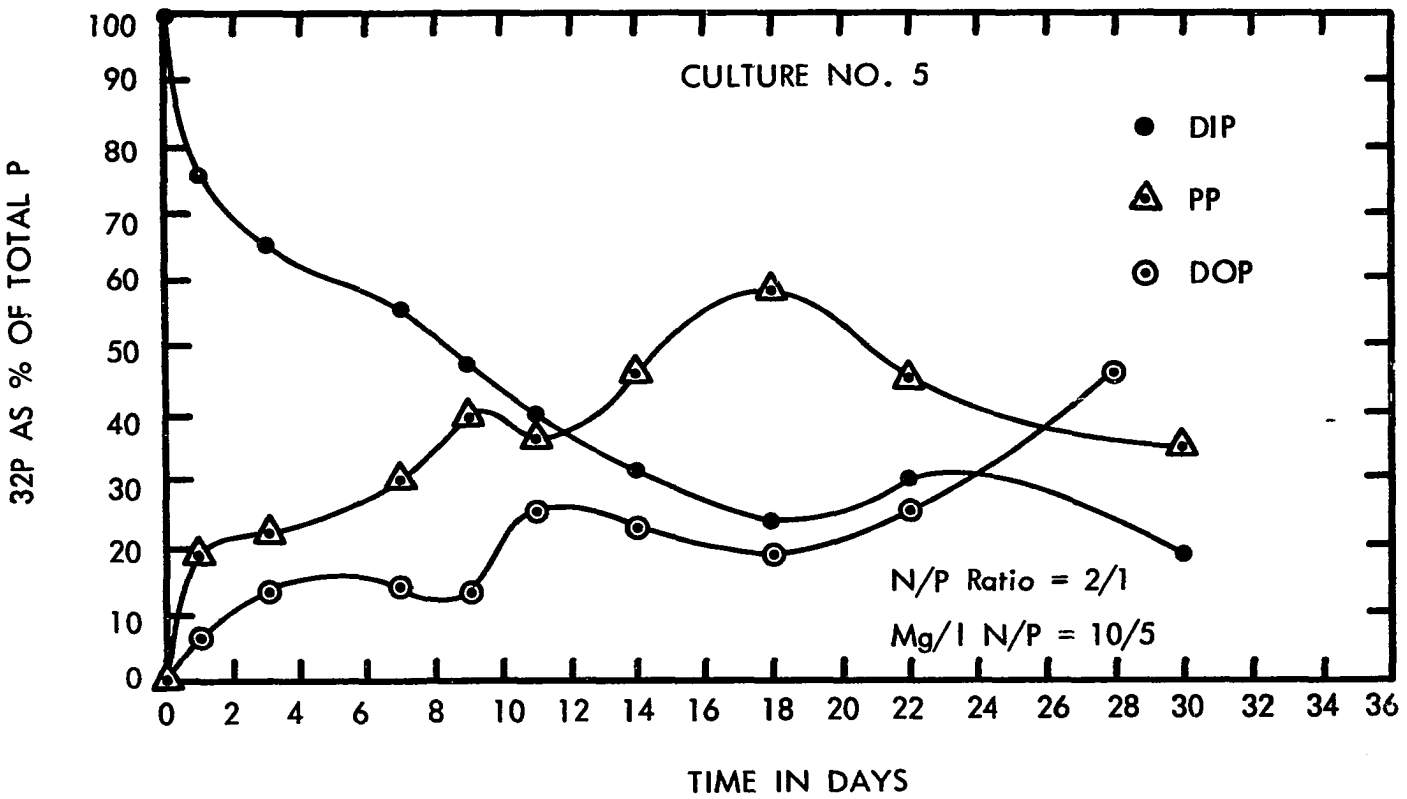


Figure 26. Percentage of 5.0 mg. DIP Incorporated into PP and DOP with time.

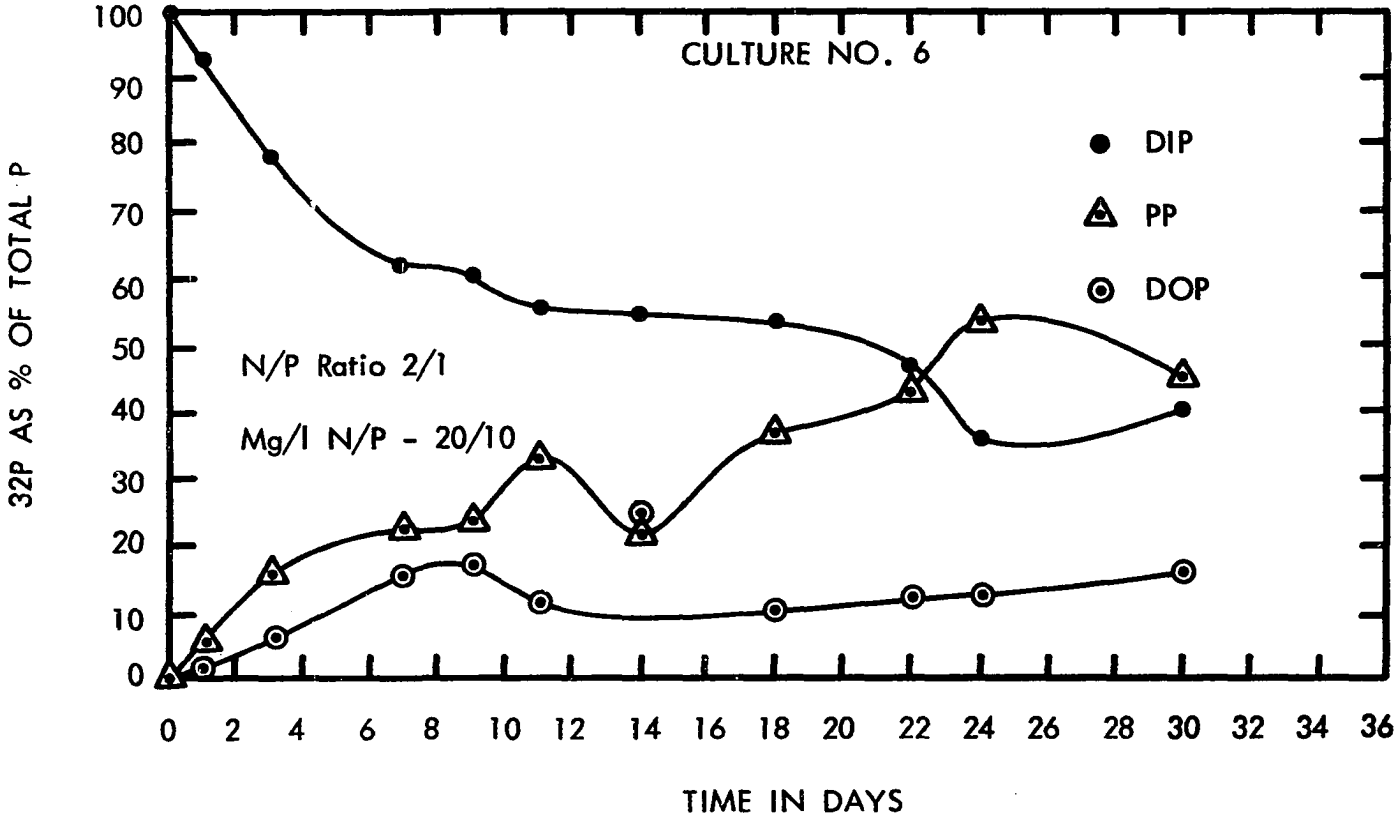


Figure 27. Percentage of 10.0 mg. DIP Incorporated into PP and DOP with time.

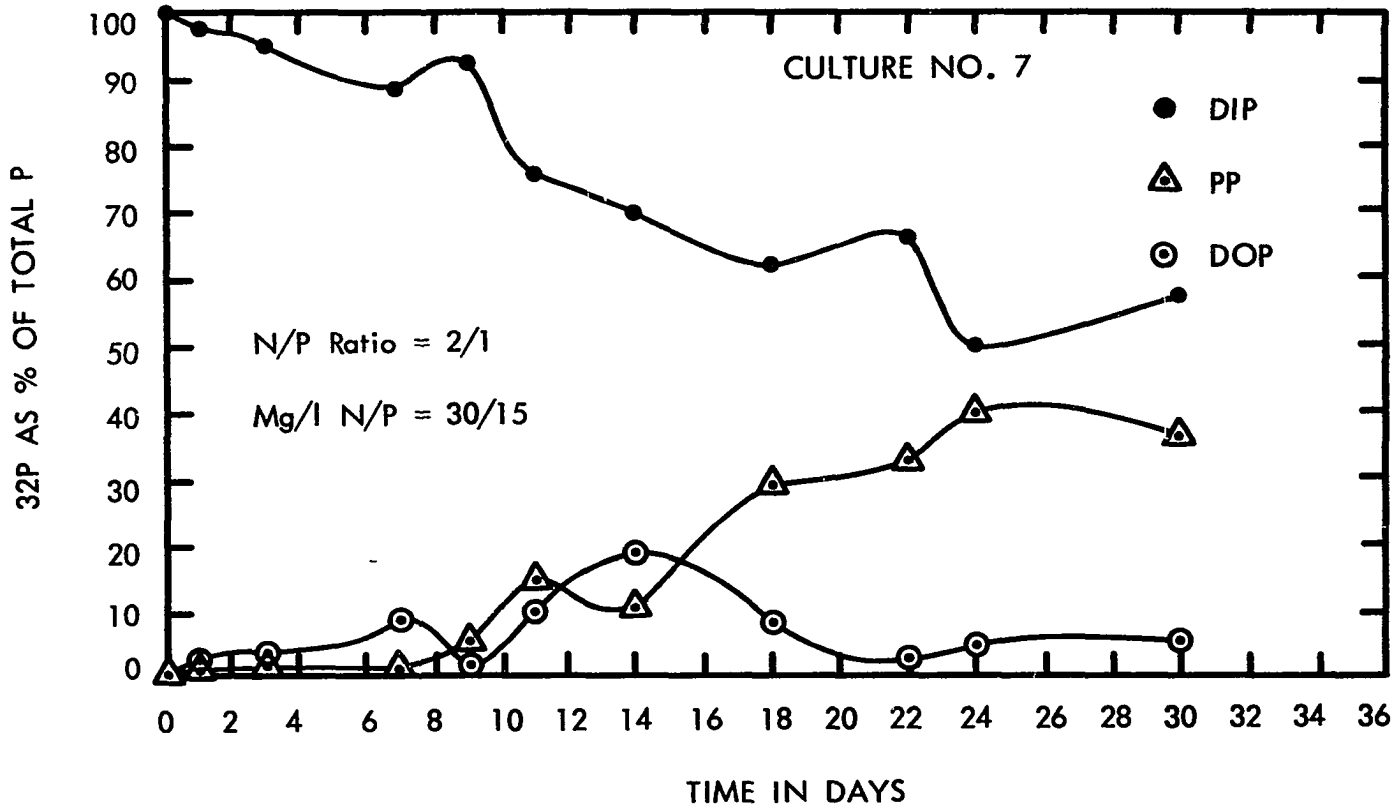


Figure 28. Percentage of 15.0 mg. DIP Incorporated into PP and DOP with time.

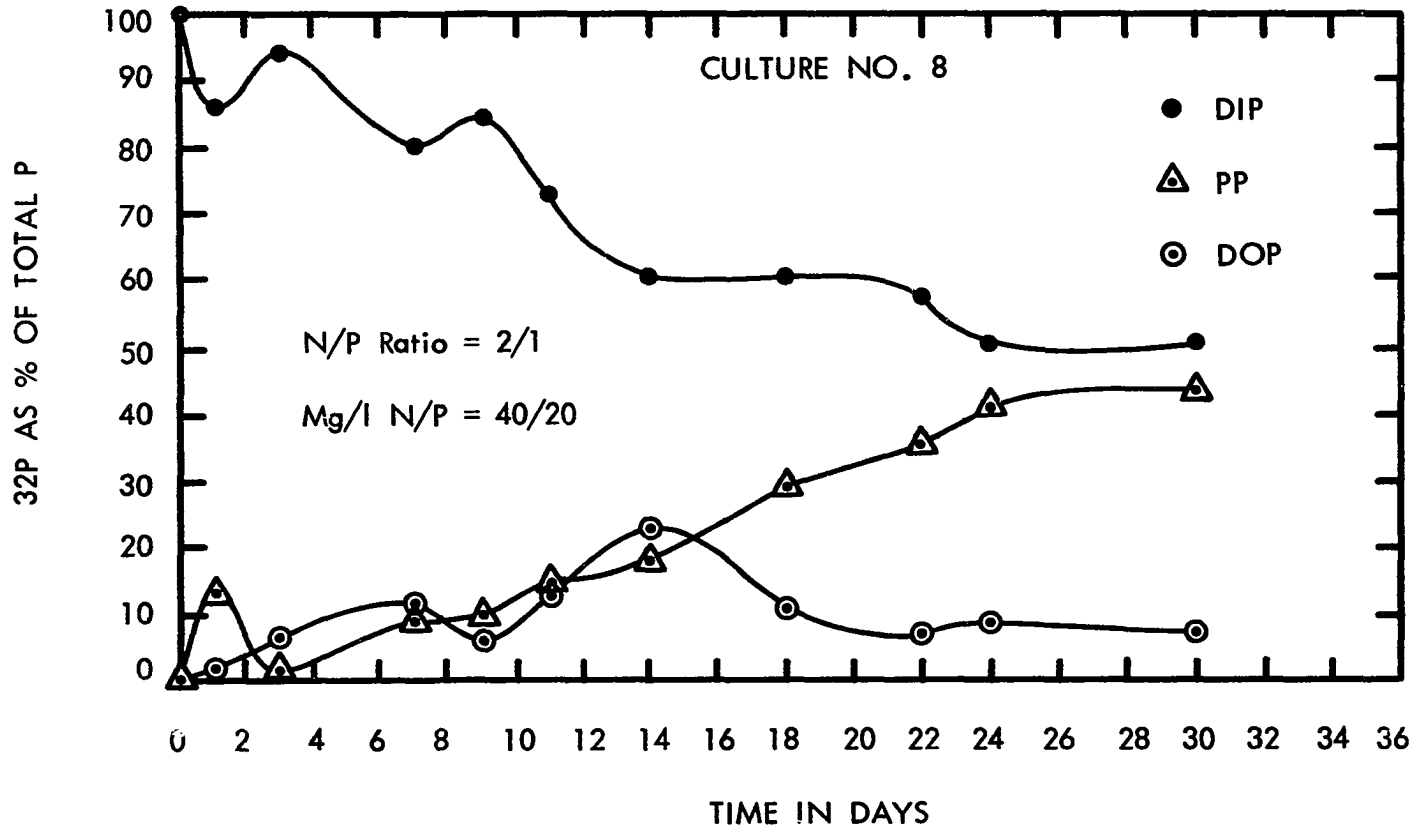


Figure 29. Percentage of 20.0 mg. DIP Incorporated into PP and DOP with time.

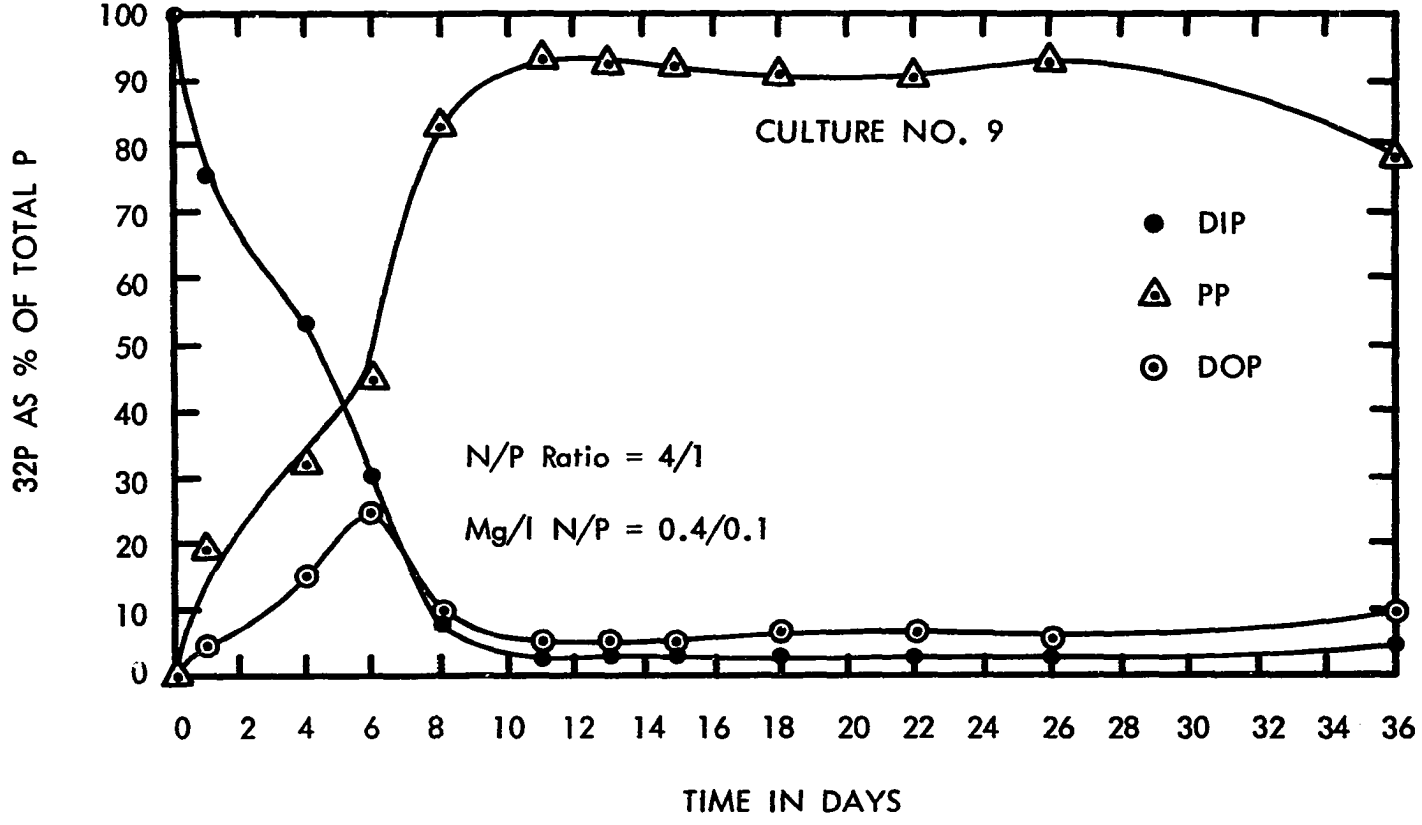


Figure 30. Percentage of 0.1 mg. DIP Incorporated into PP and DOP with time.

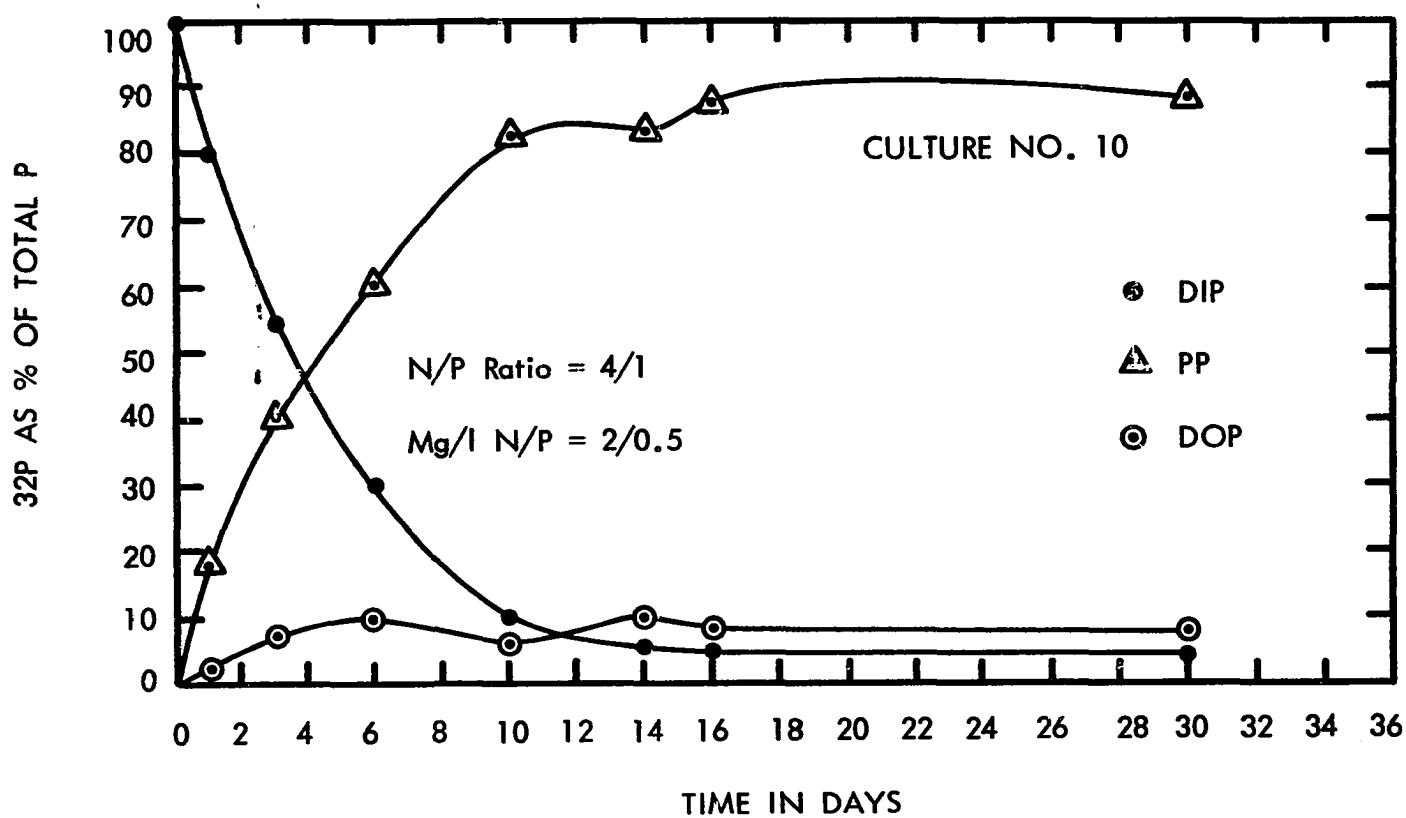


Figure 31. Percentage of 0.5 mg. DIP Incorporated into PP and DOP with time.

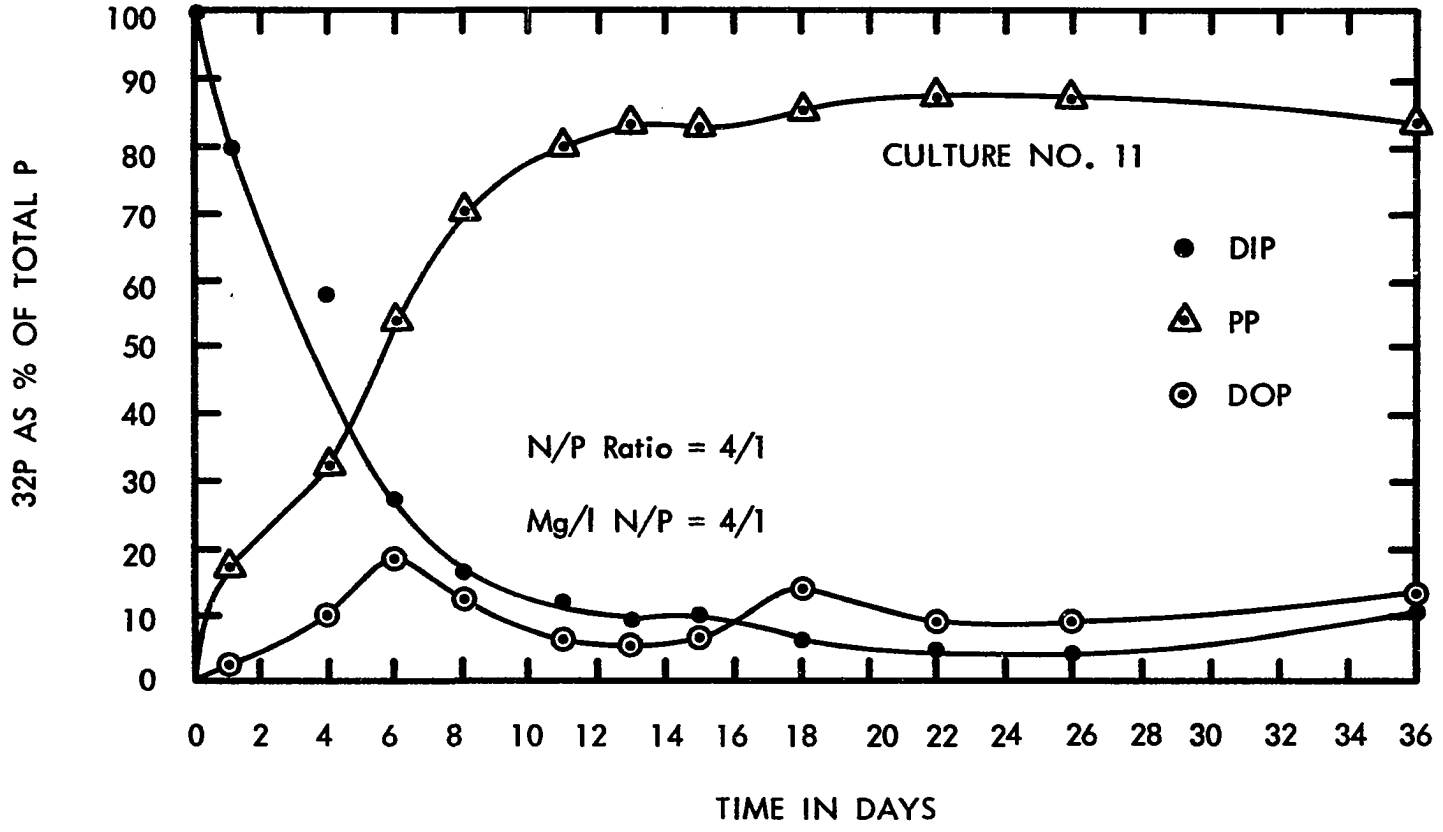


Figure 32. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

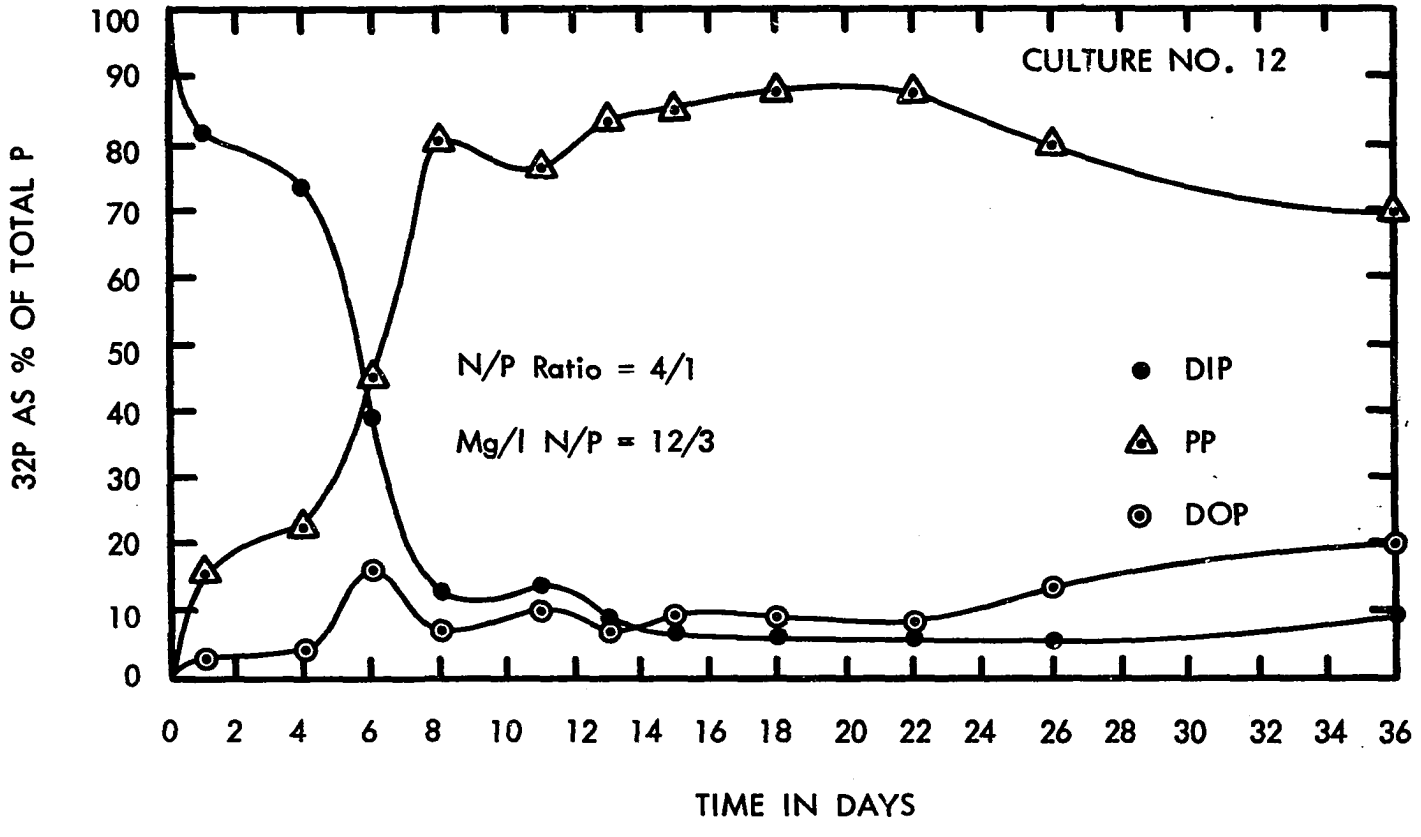


Figure 33. Percentage of 3.0 mg. DIP Incorporated into PP and DOP with time.

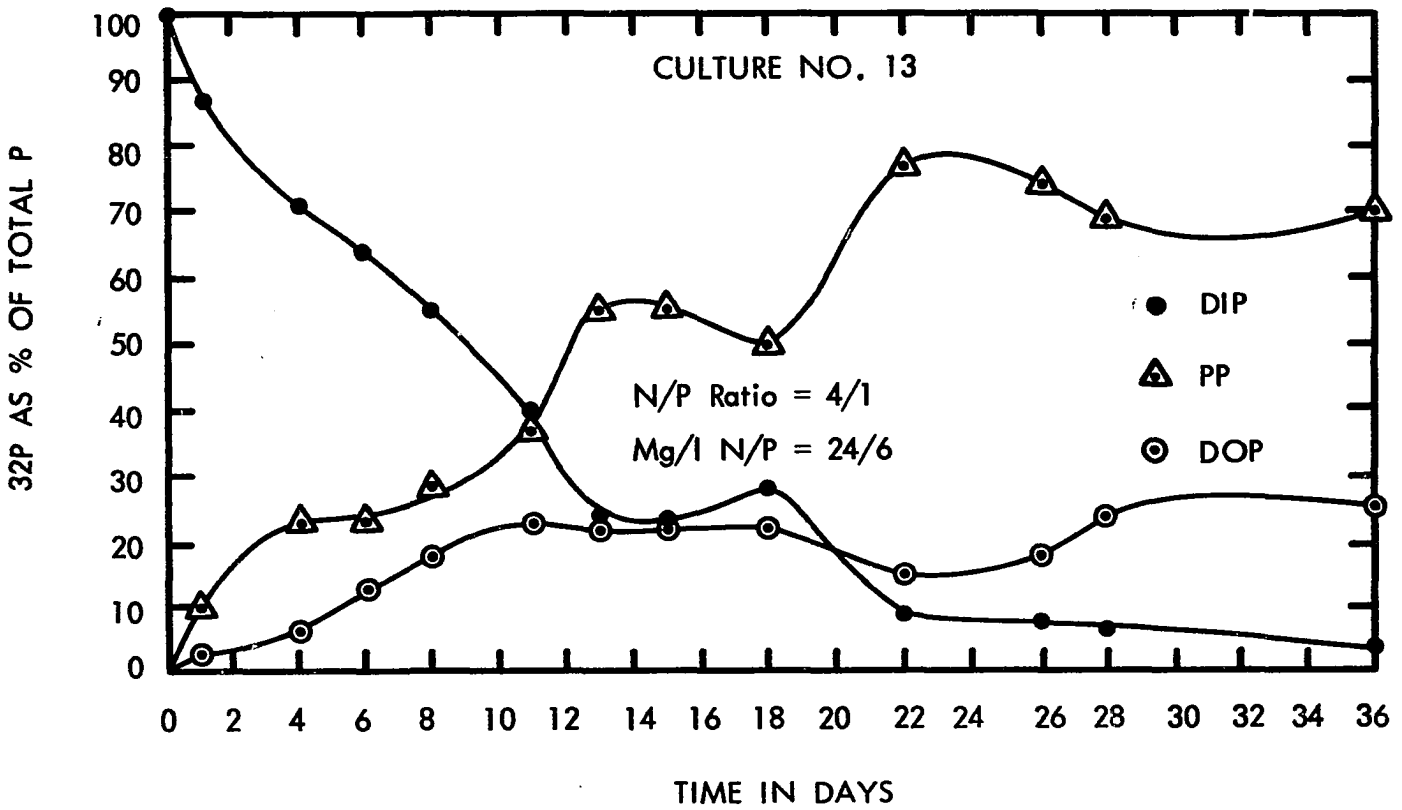


Figure 34. Percentage of 6.0 mg. DIP Incorporated into PP and DOP with time.

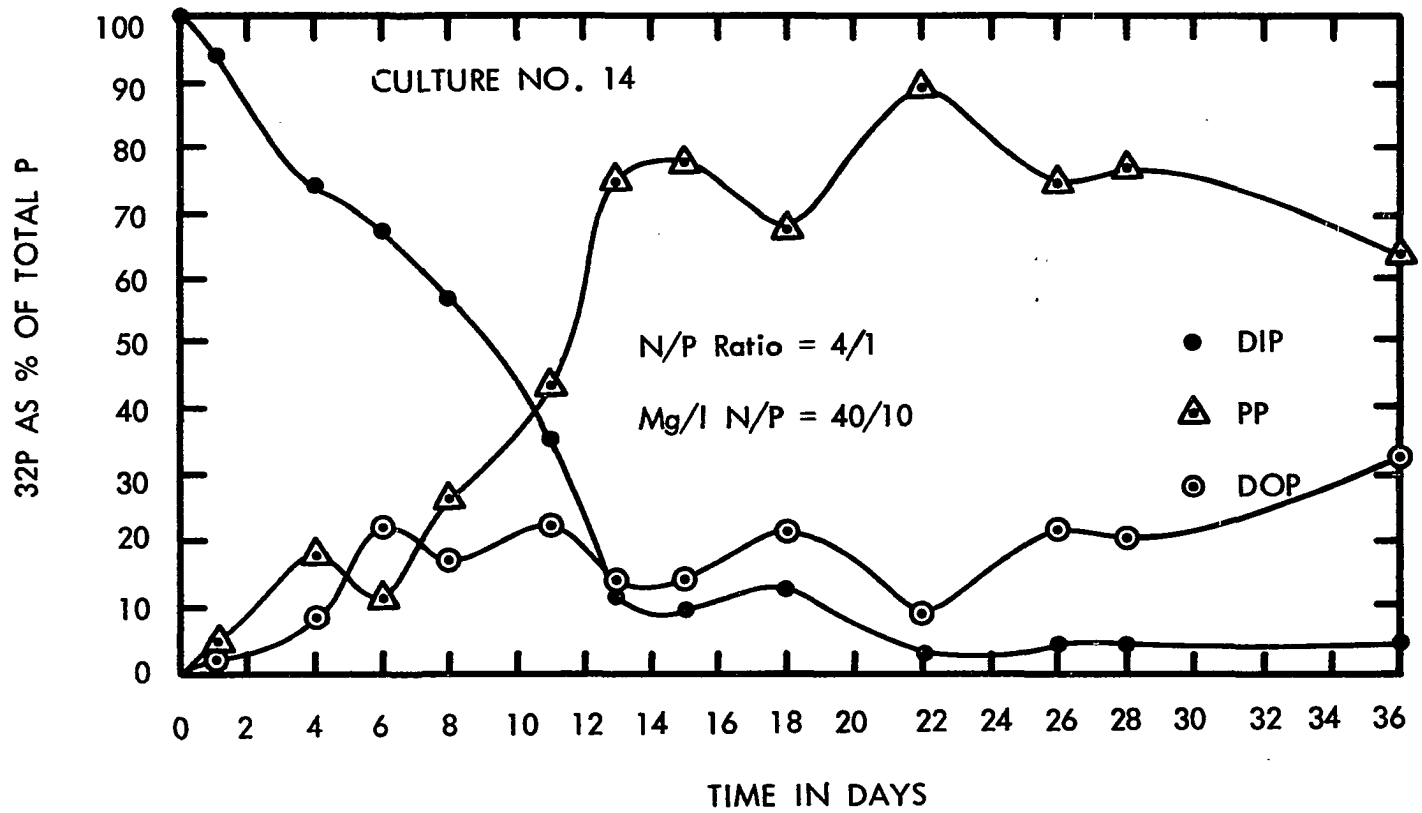


Figure 35. Percentage of 10.0 mg. DIP Incorporated into PP and DOP with time.

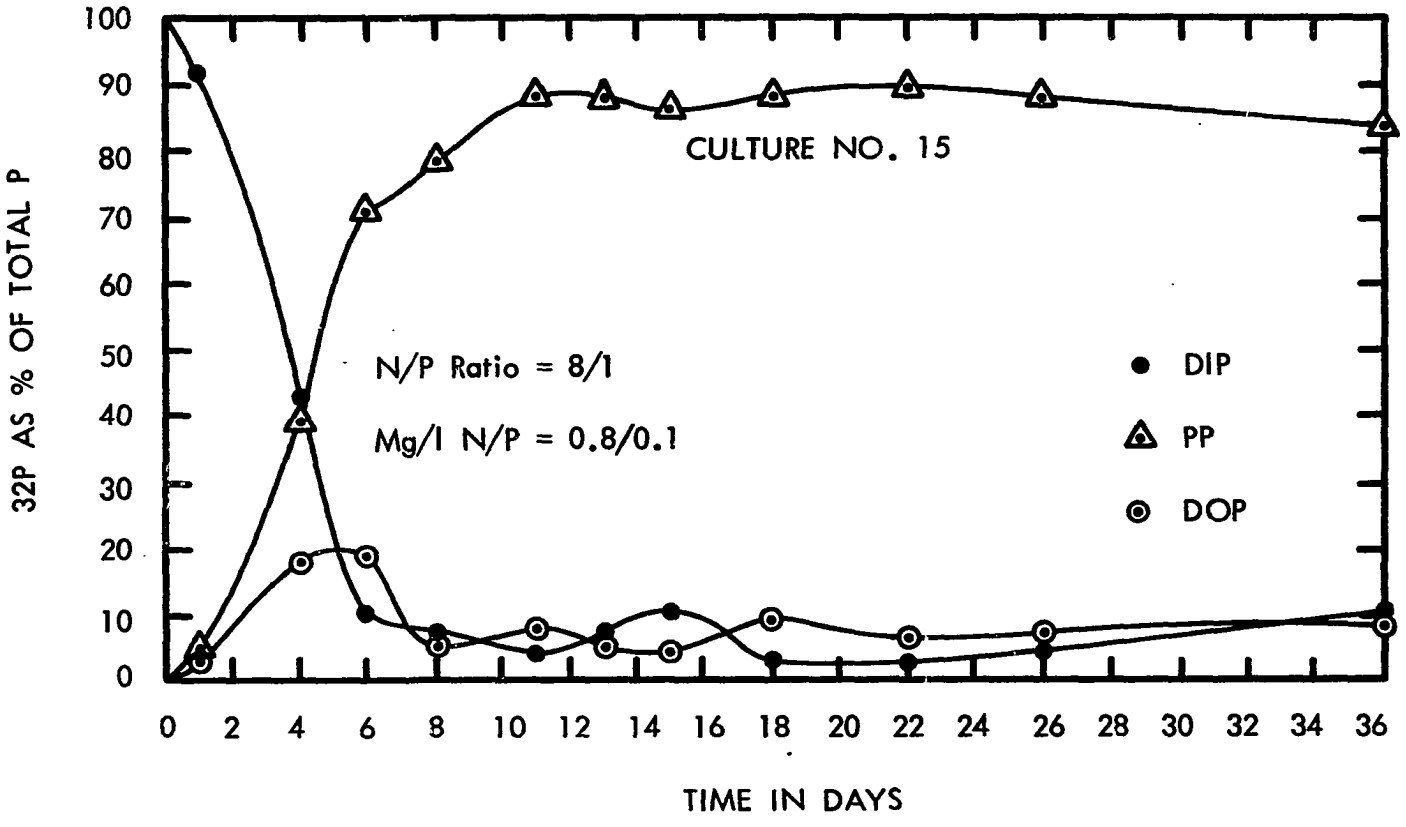


Figure 36. Percentage of 0.1 mg. DIP Incorporated into PP and DOP with time.

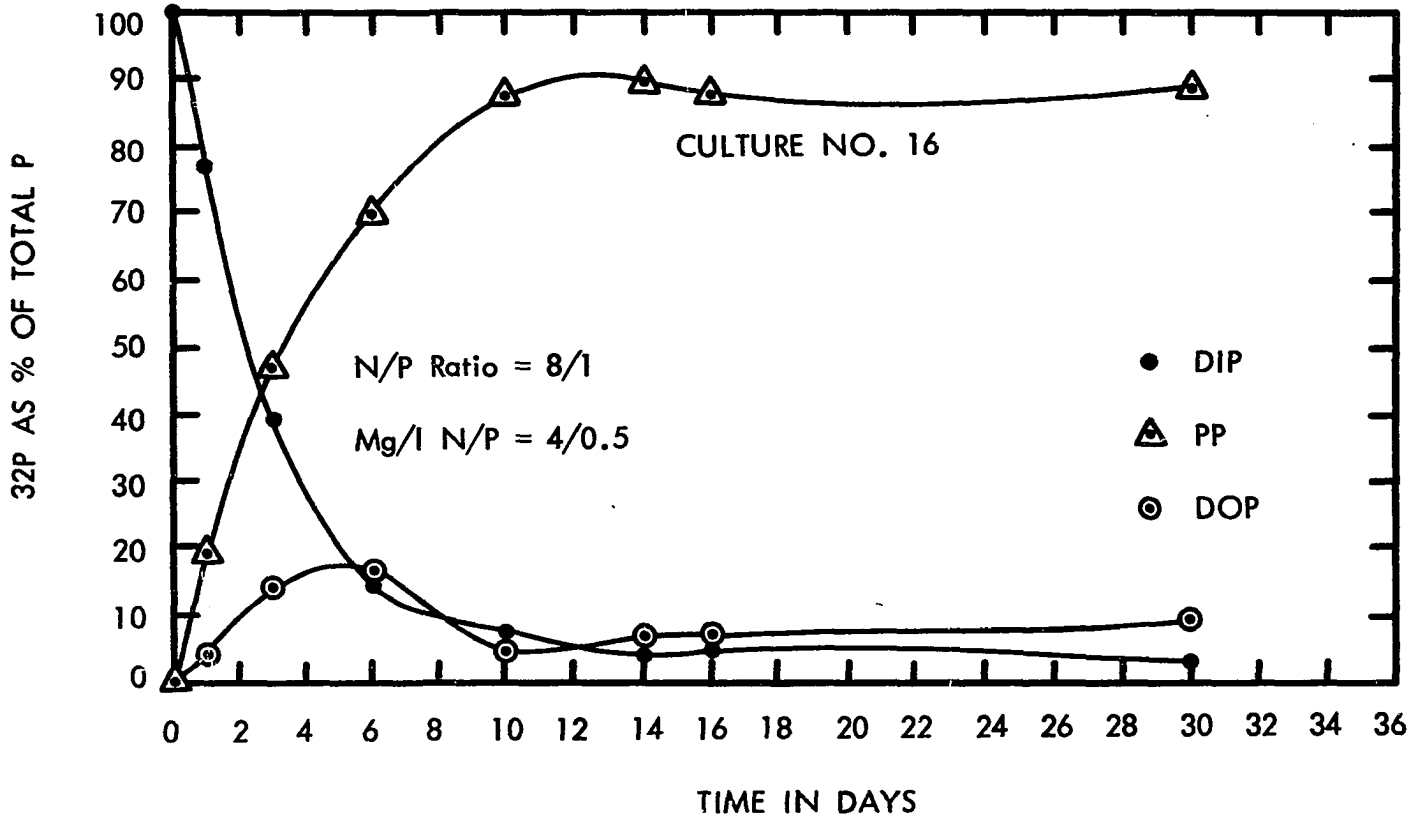


Figure 37. Percentage of 0.5 mg. DIP Incorporated into PP and DOP with time.

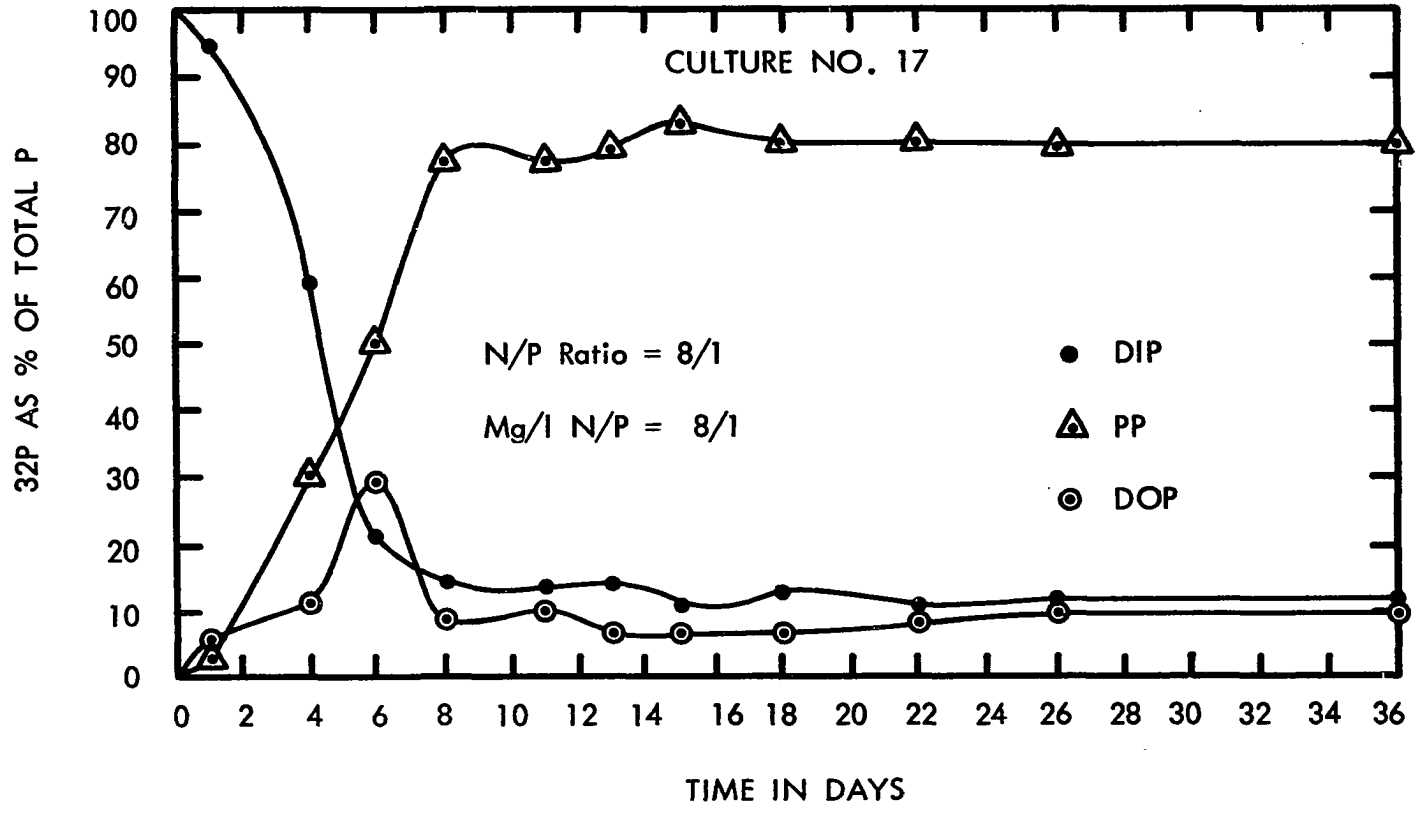


Figure 38. Percentage of 1.0 mg. DIP Incorporated into PP and DOP with time.

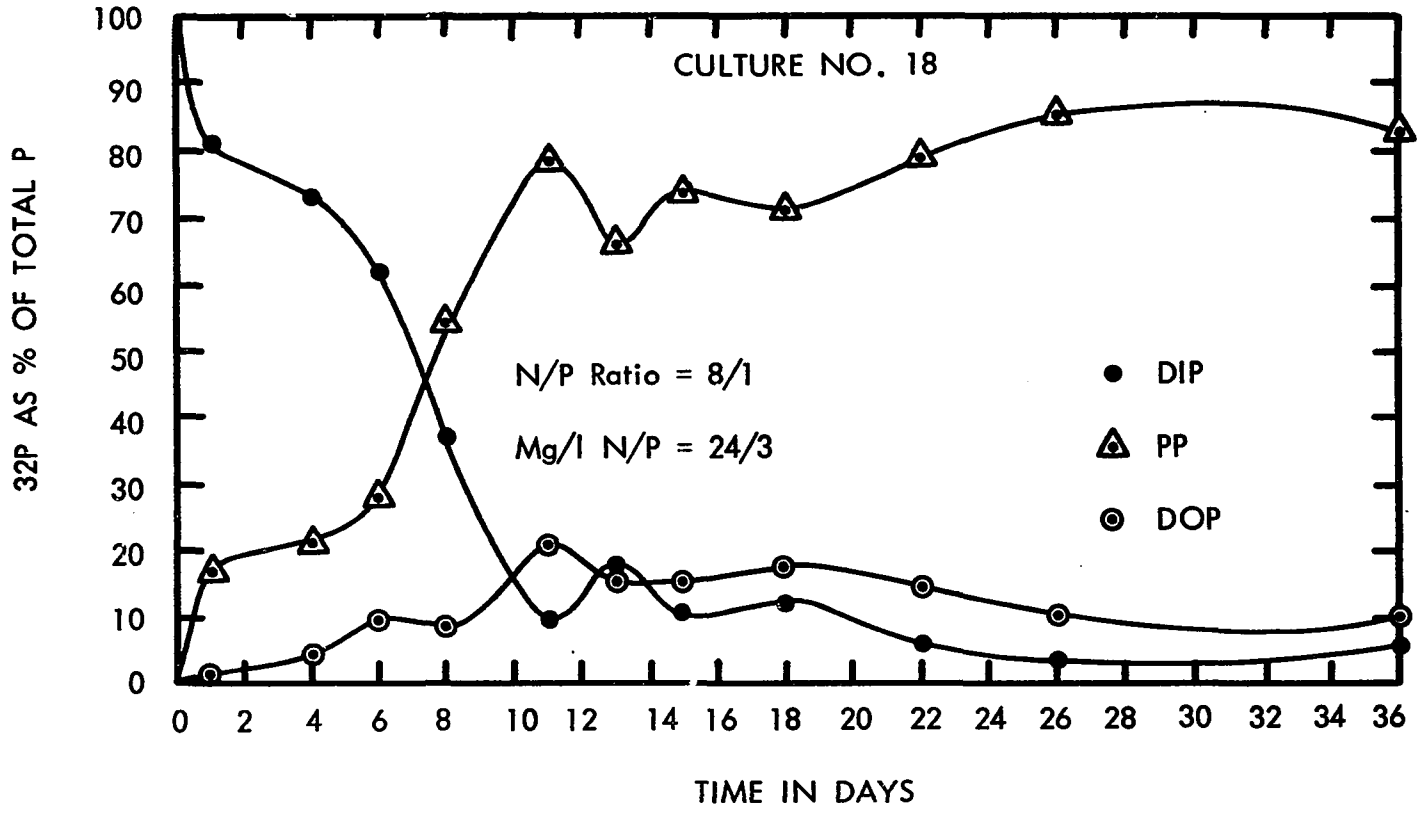


Figure 39. Percentage of 3.0 mg. DIP Incorporated into PP and DOP with time.

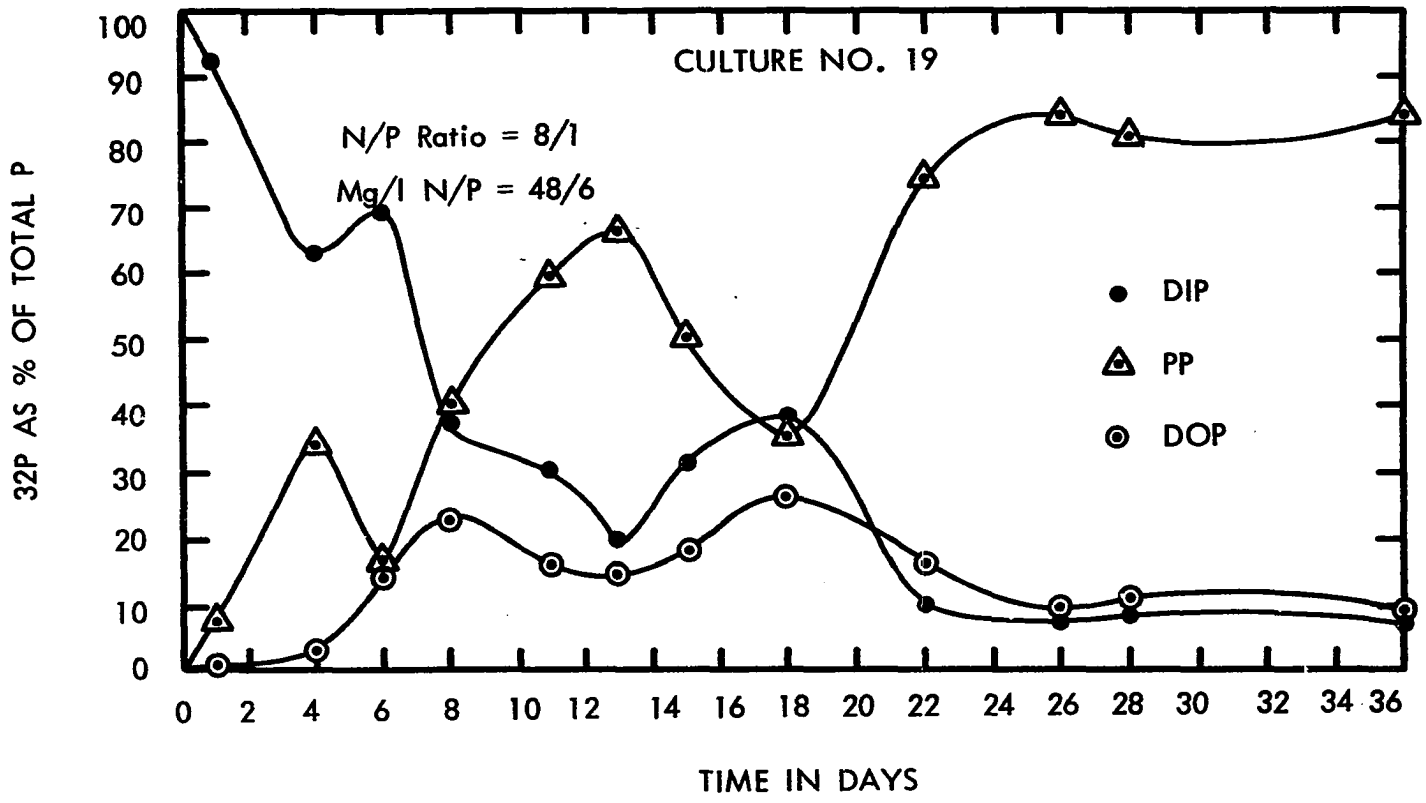


Figure 40. Percentage of 6.0 mg. DIP Incorporated into PP and DOP with time.

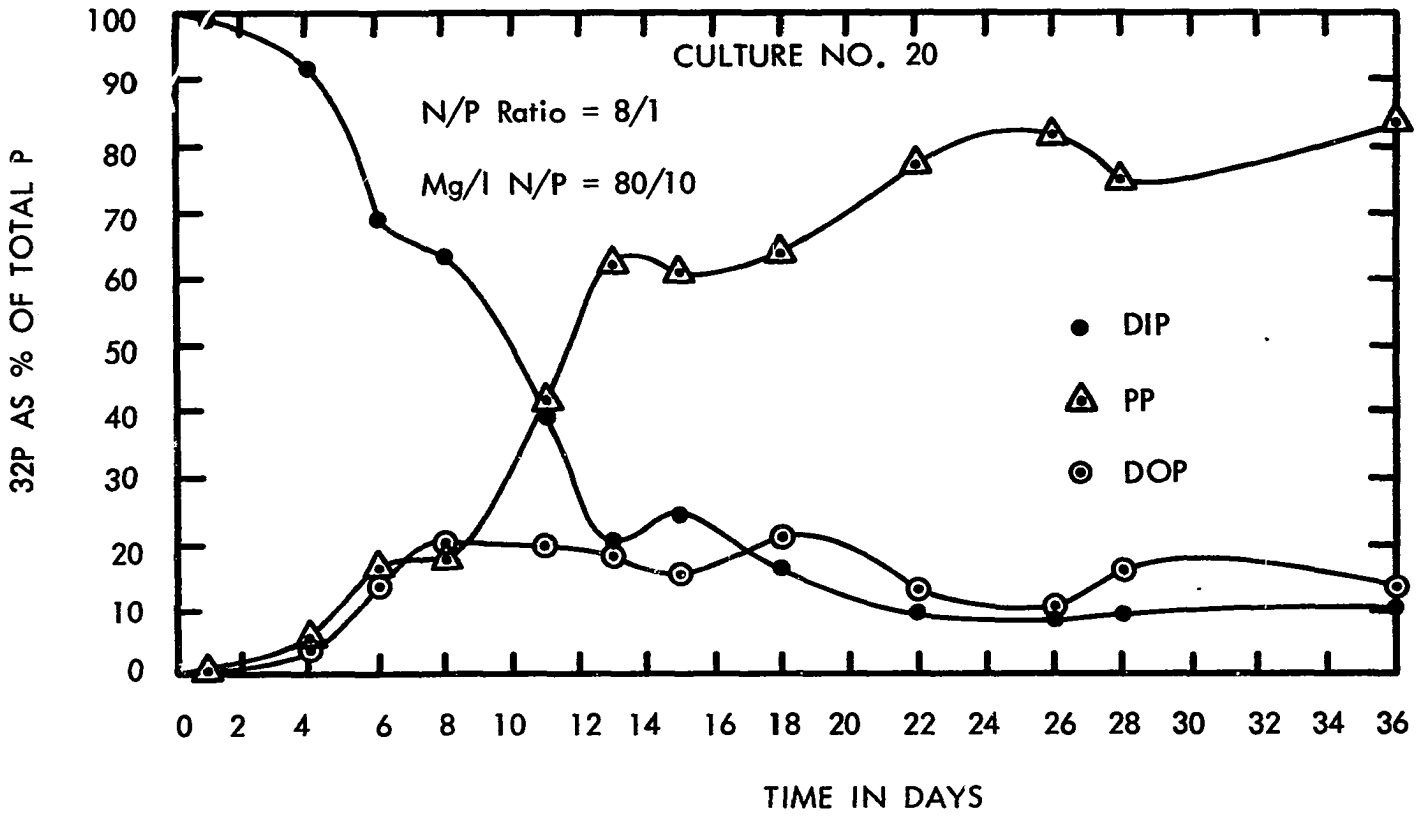


Figure 41. Percentage of 10.0 mg. DIP Incorporated into PP and DOP with time.

TABLE V

STEADY STATE PHOSPHORUS CONCENTRATIONS, EXCHANGE RATES, AND ALGAE CONCENTRATIONS RESULTING FROM VARIOUS NITROGEN AND PHOSPHORUS RATIOS AND CONCENTRATIONS

Culture No.	N/P Ratio	P. Conc. (mg/l P.)	Steady State P. Conc. (mg/l P.)			Exchange Rates (day^{-1})				Max. Algae Conc. (mg/100 ml algae)
			DIP	PP	DOP	a	b_1	b_2	c	
1	1/1	5.0	2.7	1.4	.9	.05	.10	.08	.12	170
2	1/1	10.0	4.6	4.9	.5	.06	.05	.03	.24	185
3	1/1	15.0	8.5	4.2	2.3	.05	.10	1.3	2.52	225
4	1/1	20.0	12.0	4.8	3.2	.04	.1	.16	.24	250
5	2/1	5.0	1.3	2.3	1.4	.09	.05	.08	.12	180
6	2/1	10.0	4.0	4.6	1.4	.06	.05	.07	.24	250
7	2/1	15.0	8.9	5.2	.9	.06	.10	.03	.16	290
8	2/1	20.0	10.8	7.4	1.8	.04	.06	.03	.13	315
9	4/1	0.1	.004	.09	.006	.23	.01	.02	.32	70
10	4/1	0.5	.05	.42	.03	.19	.02	.08	.74	100
11	4/1	1.0	.1	.8	.1	.17	.02	.06	.47	155
12	4/1	3.0	.3	2.4	.3	.15	.02	.28	2.52	240
13	4/1	6.0	.9	3.9	1.2	.13	.03	2.88	8.91	250
14	4/1	10.0	1.1	7.3	1.6	.14	.02	1.18	5.36	310
15	8/1	0.1	.006	.087	.007	.23	.02	.04	.50	85
16	8/1	0.5	.03	.43	.04	.18	.01	.05	.60	105
17	8/1	1.0	.12	.78	.10	.20	.03	.07	.54	190
18	8/1	3.0	.3	2.3	.4	.15	.02	.1	.56	225
19	8/1	6.0	1.8	3.0	1.2	.14	.09	.08	.20	250
20	8/1	10.0	2.0	6.0	2.0	.12	.04	1.38	4.47	305

transition was irregular. The lowest DIP concentration and the highest PP concentration generally occurred between days 18 and 24. The DOP concentration, although somewhat higher, reacted similarly to the DOP concentrations obtained from lower phosphorus concentrations.

When cultures with the same phosphorus concentrations but different N/P ratios were compared, it was noted that the cultures with 4/1 and 8/1 N/P ratios took up more phosphorus than the cultures with 1/1 and 2/1 N/P ratios. Further, when cultures of the latter two ratios with identical phosphorus concentrations were compared, in every instance except one (no.'s 2 and 6) cultures with a 2/1 N/P ratio took up more phosphorus. The cultures with N/P ratios of 8/1 did not take up more phosphorus than cultures with ratios of 4/1. These results indicate that nitrogen is limiting at the 1/1 and 2/1 ratios, but not at the 4/1 and 8/1 ratios. This explanation would agree with Ketchum (13) who showed that phosphorus uptake increased as the nitrogen concentration increased. It may be, however, the N/P ratio rather than the nitrogen concentration which influences phosphorus uptake.

In view of these results, only the data obtained from cultures containing 3 mg/l P or less was utilized in developing any predictive equations. It appears from computation of exchange rates that the Watt-Hayes Model

does not produce highly predictable results under our experimental conditions with phosphorus concentrations in excess of 3 mg/l. However, results obtained from utilizing all cultures as well as cultures containing 3 mg/l P or less were reported when appropriate.

Examination of Figures 22-41 or Table V reveals, except for cultures 3 and 4, that the steady state concentrations of DIP, PP and DOP increase as the initial phosphorus concentrations increase. A correlation coefficient of .89 between the PP steady state concentrations and the initial phosphorus concentration was obtained. Analyzing only those cultures containing 3 mg/l P or less, a very high degree of correlation, .99, was achieved.

In addition, the maximum algal concentration was found to be highly correlated with both the PP steady state concentration and the initial phosphorus concentration. Correlation between maximum algal concentrations and the steady state PP concentrations gave coefficients of .89 for all the cultures and .93 for those containing 3 mg/l P or less. For all cultures, a coefficient of .73 was computed for the correlation between maximum algal concentrations and the initial phosphorus concentrations. A correlation coefficient of .93 was computed for cultures with less than 3 mg/l P.

These results clearly indicate a strong relationship

between the initial phosphorus concentration, the PP steady state concentration and the maximum algal concentration. However, because a high degree of correlation exists between the PP steady state concentrations and the initial phosphorus concentrations, it is not possible to measure their separate influences upon algal growth when used in a multiple regression analysis. Such a linear relationship among two or more explanatory variables is called multicollinearity, and is discussed in detail by Brennan (87). As a result, in an attempt to describe the relationship between the dependent variable (algal concentration) and the two independent or explanatory variables with regression analysis, one of the latter must not be used. In this case, if the initial phosphorus concentration had been partially organic phosphorus, unavailable for algal uptake, it is doubtful that such high correlation would exist between the initial phosphorus concentration and the algal concentrations. Therefore, it appears in this case the PP steady state concentration is the most appropriate independent variable to be included in a regression equation describing the relationship between the maximum algal concentration and the phosphorus concentration. For the relationship to be useful in predicting the algal growth potential based on phosphorus input, however, the relationship between the initial phosphorus concentration and the PP steady state concentration

must be described. That is, the phosphorus related algal growth potential may be predicted from a knowledge of the initial phosphorus concentration or input if the system is in steady state or if steady state can be established following the phosphorus input. The prediction is, however, a two step process requiring first a knowledge of the relationship between the initial phosphorus concentration or input and the PP steady state concentration. Then the relationship between the PP steady state concentration and the maximum algal growth must be determined.

Using data from the cultures containing 3 mg/l P or less, the following equations gave respective coefficients of determination, R^2, of .99 and .87.

$$PP = .02 + .78\ PT$$

PP = particulate steady state phosphorus concentration--mg/l P

PT = total phosphorus concentration or phosphorus input--mg/l P

$$AG = 85.69 + 66.30\ PP$$

AG = maximum algal growth--mg/100 ml

The F statistic for both analyses was highly significant at the .01 percent significance level ($F_{.01(1,7)} = 12.25$ while computed F values = 5929.42 and 39.51 respectively) thus indicating a linear relationship between the variables.

These equations imply a functional relationship between the variables under the experimental conditions previously described and at phosphorus concentrations be-

tween 3 mg/l P and .1 mg/l P. The unexplained variability in maximum algal growth, 13 percent, might be accounted for by a combination of several things including light, temperature, CO_2 concentration, pH or some other nutrient or growth factor.

Further examination and analysis of the data in Table V revealed several highly correlated variables at phosphorus concentrations of 3 mg/l and less. Among them are exchange rate "a" vs. initial phosphorus concentration, r = .85; exchange rate "b_1" vs. the DIP and PP steady state concentrations and maximum algal growth, r = .91; exchange rate "b_2" vs. the DOP and PP steady state concentrations and maximum algal growth, r = .98; and exchange rate "c" vs. DOP and PP steady state concentrations and maximum algal growth, r = .97.

As indicated previously, the exchange rate "a" should, according to the Michaelis Menten expression, be a function of the phosphorus concentration as long as phosphorus is limiting. This relationship appears to be valid for the cultures containing 3 mg/l P or less.

Strong correlation existed between "b_1" and the PP and DIP steady state concentrations and between "b_2" and the PP and DOP steady state concentrations. This was to be expected since all the exchange rates were correlated with the initial phosphorus concentration which

in turn was highly correlated with the steady state concentrations. Therefore, the steady state concentrations directly involved with a particular reaction were used along with maximum algal concentrations to predict exchange rates.

Predictive equations for the various exchange rates were obtained from regression analyses. They and the coefficients of determination follow:

$$a = 213.42 - 2.25\ PT$$

$$R^2 = .73$$

a = exchange rate "a"--days^{-1}

PT = total phosphorus concentration or phosphorus input--mg/l P

$$b_1 = .01 + .0001\ AG - .04\ PP + .28\ DIP$$

$$R^2 = .82$$

b_1 = exchange rate "b_1"--days^{-1}

AG = maximum algal growth--mg/100 ml

PP = particulate steady state phosphorus concentration--mg/l P

DIP = dissolved inorganic steady state phosphorus concentration--mg/l P

$$b_2 = - .025 - .0003\ AG + .31\ PP - 1.43\ DOP$$

$$R^2 = .95$$

b_2 = exchange rate "b_2"--days^{-1}

DOP = dissolved organic steady state phosphorus concentration--mg/l P

$$c = .48 - .005\ AG + 3.30\ PP - 15.69\ DOP$$

$R^2 = .94$

c = exchange rate "c"--$days^{-1}$

As is readily seen, these equations do not fully explain all the variation in the dependent variables. In all cases, however, the F statistic was significant, implying a linear relationship between the variables. Errors in the regression analyses are errors of omission rather than errors of incorporation which in this study often would have resulted in multicollinearity. Every exchange rate was significantly correlated with either the initial phosphorus concentration or the PP steady state concentration. Because of the linear relationship between these variables they cannot both be used as independent variables in the same equation. Therefore, the one contributing least to the correlation coefficient, the initial phosphorus concentration, was deleted.

By being able to predict the exchange rates, insight into the time necessary for an aquatic system to reach steady state following a phosphorus input may be gained. At any rate, the ability to accurately predict phosphorus exchange rates will allow one to more realistically view the complicated eutrophication process.

The predictive equations presented in this study will not necessarily apply to real systems; but because algal growth and exchange rates can be predicted with some degree of accuracy in the laboratory, it is conceivable that they could be predicted for the field as well.

SUMMARY AND CONCLUSIONS

In the relationship of nutrients to eutrophication there are at least two variables that demand attention, with the implication that one, algal growth, is dependent on the other, phosphorus. There is little doubt that this relationship exists but to what extent and under what conditions remains a mystery.

This laboratory study was undertaken as an effort to provide information which might lead to the establishment of defined relationships existing between phosphorus and eutrophication.

The applicability to eutrophication of the Watt-Hayes Steady State Model describing phosphorus transfer and exchange has been determined for the selected conditions of this study. By determining inorganic, particulate and organic steady state phosphorus concentrations and exchange rates resulting from various phosphorus inputs, equations enabling one to predict exchange rates, steady state phosphorus concentrations and maximum algal growth have been developed.

Phosphorus concentrations ranged from .1 mg/l P to 20 mg/l P and N/P ratios varied from 1/1 to 8/1. Data from cultures with N/P ratios of 1/1 or 2/1 and from cultures containing more than 3 mg/l P were used to calculate steady state phosphorus concentrations and exchange rates. They were not used in the development of the predictive equations because the Watt-Hayes Model did not produce highly predictable results at these ratios and concentrations. It was found that above 3 mg/l, algae stored significant amounts of phosphorus. This probably accounted for the poorly established steady state conditions and poorly correlated exchange rates in cultures with phosphorus concentrations in excess of 3 mg/l P.

High correlation (r = .99) was found between the initial phosphorus concentrations and the resulting particulate steady state phosphorus concentrations, and between the particulate steady state phosphorus concentrations and the maximum algal growth attained (r = .93). Because multicollinearity existed between the particulate steady state phosphorus concentration and the initial phosphorus concentration, these two explanatory variables could not be used in the same regression analysis. Therefore, prediction of the maximum algal growth is a two step process as follows:

$$PP = .02 + .78\ PT\ (R^2 = .87)$$

PP = particulate steady state phosphorus concentration--mg/l P

PT = total phosphorus concentration or phosphorus input--mg/l P

$$AG = 85.69 + 66.30\ PP \quad (R^2 = .87)$$

AG = maximum algal growth--mg/100 ml

The F statistic for both analyses was highly significant.

Other highly correlated variables from which predictive equations were developed include exchange rate "a" and the initial phosphorus concentration, r = .85; exchange rate "b_1", the DIP and PP steady state concentrations and the maximum algal concentrations, r = .91; exchange rate "b_2", the DOP and PP steady state concentrations and the maximum algal concentrations, r = .98; and exchange rate "c", the DOP and PP steady state concentrations and the maximum algal concentrations, r = .97.

Equations which may be used for predicting exchange rates and their coefficients of determination follow:

$$a = 213.42 - 2.25\ PT$$

$$R^2 = .73$$

a = exchange rate "a"--$days^{-1}$

PT = total phosphorus concentration or phosphorus input--mg/l P

$$b_1 = .01 + .0001\ AG - .04\ PP + .28\ DIP$$

$$R^2 = .82$$

b_1 = exchange rate "b_1"--$days^{-1}$

AG = maximum algal growth--mg/100 ml

PP = particulate steady state phosphorus concentration--mg/l P

DIP = dissolved inorganic steady state phosphorus concentration--mg/l P

$$b_2 = - .025 - .0003\ AG + .31\ PP - 1.43\ DOP$$

$$R^2 = .95$$

b_2 = exchange rate "b_2"--days^{-1}

DOP = dissolved organic steady state phosphorus concentration--mg/l P

$$c = .48 - .005\ AG + 3.30\ PP - 15.69\ DOP$$

$$R^2 = .94$$

c = exchange rate "c"--days^{-1}

It should be re-emphasized that all the equations developed from this study are valid only for data obtained under the experimental conditions of this investigation. Because of the success enjoyed in the laboratory, and because of the relative simplicity of the model, its manipulation and the determination of its inputs, it appears that similar investigations could be successful under actual circumstances. To be truly representative, however, factors such as sediment-water interchange, littoral vegetation and perhaps other physical, chemical or biological processes should be considered.

A final analysis of this study indicates the concentration of inorganic and organic phosphorus is maintained at a level at which the rate of uptake by plankton is

balanced by the rate of loss from plankton and no increase in particulate phosphorus is possible unless there is an external phosphorus input. When inorganic or organic phosphorus is added to the cultures, the rates of loss and uptake by plankton are increased and new steady state phosphorus concentrations result. In view of this, eutrophication might be defined as the addition of phosphorus in concentrations great enough to prevent the establishment of steady state conditions. The phosphorus concentrations necessary to produce eutrophication would vary from system to system, but eutrophication would be defined for a particular system in finite terms.

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APPENDIX A

STEADY STATE AND RATE OF CHANGE EQUATIONS

Steady state model:

$$DIP \underset{b_1}{\overset{a}{\rightleftharpoons}} PP \underset{c}{\overset{b_2}{\rightleftharpoons}} DOP$$

Rate of change equations describing the model:

$$\frac{d(DIP)}{dt} = -a(DIP) + b_1(PP)$$

$$\frac{d(DOP)}{dt} = -c(DOP) + b_2(PP)$$

Setting the derivatives equal to zero results in the following steady state equations:

$$a(DIP) = b_1(PP)$$

$$c(DOP) = b_2(PP)$$

For a finite time interval the rate of change equations are written:

$$\frac{(DIP)_2 - (DIP)_1}{\Delta t} = -a\frac{(DIP)_1 + (DIP)_2}{2} + b_1\frac{(PP)_1 + (PP)_2}{2}$$

$$\frac{(DOP)_2 - (DOP)_1}{\Delta t} = -c\frac{(DOP)_1 + (DOP)_2}{2} + b_2\frac{(PP)_1 + (PP)_2}{2}$$

APPENDIX B

SAMPLE EXCHANGE RATE COMPUTATION

Steady state values: DIP = 10%, PP = 80%, DOP = 10%

Values at day 0: DIP = 100%, PP = 0%, DOP = 0%

Values at day 9: DIP = 12%, PP = 80%, DOP = 8%

$$a(DIP) = b_1(PP) \qquad c(DOP) = b_2(PP)$$
$$a(.10) = b_1(.80) \qquad c(.10) = b_2(.80)$$
$$a = 8b_1 \qquad c = 8b_2$$

$$\frac{(DIP)_2 - (DIP)_1}{9} = -a\frac{(DIP)_1 + (DIP)_2}{2} + b_1\frac{(PP)_1 + (PP)_2}{2}$$

$$\frac{.12 - 1.00}{9} = -a\frac{1.00 + .12}{2} + b_1\frac{0 + .80}{2}$$

$$-.098 = -8b_1(.56) + b_1(.40)$$
$$-.098 = -4.48b_1 + .40b_1$$
$$b_1 = .024 \text{ days}^{-1}$$
$$a = 8b_1 = .192 \text{ days}^{-1}$$

$$\frac{(DOP)_2 - (DOP)_1}{2} = -c\frac{(DOP)_1 + (DOP)_2}{2} + b_2\frac{(PP)_1 + (PP)_2}{2}$$

$$\frac{.08 - 0}{9} = -c\frac{0 + .08}{2} + b_2\frac{0 + .80}{2}$$

$$.009 = -8b_2(.04) + b_2(.40)$$
$$.009 = -.32b_2 + .40b_2$$
$$b_2 = .113 \text{ days}^{-1}$$
$$c = 8b_2 = .904 \text{ days}^{-1}$$