ASSESSEMENT OF THE BIOLOGICAL AVAILABILITY OF PARTICULATE-PHASE PHOSPHORUS

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ABSTRACT

A bioassay procedure for particulate-phase phosphorus (P), using Anabaena subcylindrica was developed and evaluated. Measurements of chlorophyll concentration and whole cell alkaline phosphatase activity were established as reliable indices of biomass and algal P status, respectively. Algal P content was found to be dependent on external P availability and was directly related to biomass, only when P availability was constant. The availability of P to Anabaena was controlled by culturing Anabaena in systems containing P sorbed on hydrous ferric oxide gel, saturated to varying proportions of the sorption maximum. By manipulating the amounts of P and gel, algae of similar P status to those grown in soil systems were produced.

The combined bioassay-chemical fractionation procedure developed was used to chemically characterize the amounts and forms of biologically-available particulate inorganic P (IP) and organic P (OP) in potential surface runoff fractions from a wide range of soils. The simultaneous fractionation of Anabaena of similar P status to those in the bioassay systems, enabled a correction to be made for the algal-P contribution to extractable soil + algal P. In this way, the depletion of particulate IP and OP could be monitored. Algal growth depleted P from the 0.1M NaOH-soil-P fraction only; in several bioassays, 0.1M NaOH-soil-OP constituted the larger part of the P depleted. For most of the materials studied, except allophanic material, 0.1M NaOH-soil-P was depleted by 70 to 100% during the growth of Anabaena. Extractability in 0.1M NaOH suggests that biologically-available IP is present as surface-sorbed IP. A similar origin is probable for particulate-phase OP.

The amounts of particulate P extracted by persulphate digestion, a

commonly-used extraction procedure, were greater than those of biologically-available particulate P. Conversely, the amounts of isotopically-exchangeable P underestimated those of biologically-available particulate IP, as determined by the developed procedure.

Algal-soil contact was an important factor influencing the depletion of soil P. Soluble, algal-extracellular products, acting in isolation from the algae, had little influence on particulate IP desorption. Also, the simple desorption of particulate IP was unable to account for the release of large amounts of P to the algae. The initial solution P concentration maintained by a particulate P source material considerably influenced the amount of algal growth and the extent to which particulate P was subsequently depleted.

Biologically-available OP in two soil materials was extracted with 0.1M NaOH and the extracts were separated into humic and fulvic material, which were fractionated by agar gel and Sephadex gel chromatography, respectively. Most of the OP in the humic extract was present as high molecular weight organic matter-Fe-P complexes. In the fulvic extract, both high and low molecular weight organic matter-Fe-P complexes were identified. Inositol polyphosphates, both free and complexed, were identified by ion-exchange chromatography in the fulvic material.

A major objective of the present study was the development of a chemical test for estimating the amount of biologically-available P in stream sediment-source materials. Except for samples containing allophanic material, the extraction of a water sample with 0.1M NaOH is proposed as a rapid and simple test for estimating the maximum amount of biologically-available P present in the sample.

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SECTION 1

INTRODUCTION

The excessive growth of algae and other aquatic plants has an obvious adverse effect on the recreational value of waters (Lund, 1972). This feature and the deterioration in quality of domestic-water supplies, have prompted considerable research into the causes of increased biological productivity in waters. Evidence from these studies indicates that external inputs of plant nutrients are invariably the major controlling factor. In many waters, phosphorus (P) has been identified as the "key" growth-limiting nutrient. Consequently, inputs of biologically-available P may be expected to have a detrimental effect on water quality. This is now well recognized and in many cases the extent of algal production has directly been related to the input of available P.

In terms of controlling the amounts of available P entering waters, diffuse sources such as particulate P derived from rural and urban runoff, present a much greater problem than point sources such as sewage effluent. These diffuse sources can contribute large amounts of total P entering waters. A varying, but often substantial part of the particulate inorganic P (IP) and organic P (OP) is not immediately biologically available to algae. It has been suggested (Lee, 1973) that the fractions of particulate IP and OP that become available in natural waters are extremely important in designing meaningful control programmes for diffuse sources.

The forms and amounts of particulate IP and OP can be determined empirically by chemical extraction. In the case of IP, the relative availability of the fractions can be estimated by sorption-desorption and isotopic exchangeability studies. The biological availability of particulate

P can be assessed directly by more time-consuming, algal bioassay procedures. Further, by the chemical fractionation of particulate P before, during, and after the growth of algae, i.e., by using a combined bioassay-P fractionation procedure, it may be possible to establish the amounts and forms of biologically-available particulate IP and OP if an accurate correction for the contribution of algal P to the amount of P extracted can be made.

Although such a combined procedure would provide useful information, it would be very time consuming, thus making the characterization of biologically-available IP and OP in a large number of samples a laborious task. By the careful selection of a chemical extraction procedure it may be possible to exclusively extract the fractions of particulate IP and OP that consistently exhibit high biological availability. The chemical extraction procedure could then provide a rapid method for estimating the amount of biologically-available P in particulate materials. This method would be more convenient for the routine analysis of large numbers of diffuse runoff source materials, in order to assess their potential impact on water quality.

The initial purpose of this study was to characterize the amounts and forms of biologically-available P in materials which contribute, actually and potentially, to stream-sediment loads, by the development of an effective bioassay-P fractionation procedure. By studying a range of particulate materials, the aim was to select a chemical extraction procedure that could be used routinely to measure the amounts of biologically-available P in particulate materials. It was also intended to investigate the mechanisms which influence the biological availability of particulate P.

SECTION 2

LITERATURE REVIEW

2.1 Eutrophication

2.1.1 Phosphorus: a key nutrient?

Eutrophication is the nutrient enrichment of waters which results in the increased production of algae and macrophytes, and the deterioration of fisheries and water quality. These effects are undesirable in that they interfere with water uses. In undisturbed lakes, eutrophication is a natural aging process which eventually terminates in the disappearance of the lake itself. This process may be accelerated greatly by man's activities, producing rapid changes in water quality and the increased growth of algae in surface waters.

It is well established that phosphorus (P) is an important nutrient which may limit algal growth in natural waters (Ohle, 1953; Vollenweider, 1968; Shapiro, 1970; Fuhs et al., 1972; Lee, 1973). Significant correlations have been found between the amount of P available and the density of algal growth in 46 Swiss lakes (Vollenweider, 1970; cited by Thomas, 1973). These findings are not suprising because P compounds play a major role in cell metabolism and, more importantly, act as stores and transport intermediates for the chemical energy needed for life processes. Since the study of Vollenweider (1970), relationships between P levels in water and algal productivity have been demonstrated for natural waters in laboratory studies (Maloney et al., 1972; Miller et al., 1974) and in field studies (Powers et al. 1972). Wood and Gibson (1973) concluded that in Lough Neagh, one of the world's most eutrophic lakes, P was the critical nutrient limiting the production of algae mainly because dissolved inorganic P (DIP) was virtually undectable in lake water samples during

periods of algal growth. In contrast, nitrate-N was present throughout the summer. In bioassays of Lough Neagh water, Parr and Smith (1976) showed that additions of N, P, and Fe stimulated algal growth. Because N limitation could be alleviated by algal fixation of atmospheric N, it was unlikely that control of N inputs would significantly decrease algal growth. Inputs of P attributable to human activity, on the other hand, could be controlled to some extent and would probably lead to reductions in the algal population, as shown by reductions in P inputs to Danish (Mathiesen, 1970) and American (Edmonson, 1970) lakes. Vollenweider (1968) produced data to show how the trophic states of a group of lakes could be related to the annual loading of P and lake depths. This relationship can be explained by simple models relating P inputs to algal levels (Vollenweider, 1969a; Dillon and Rigler, 1974; Bachman and Jones, 1974, Jones and Backman, 1976).

In a short review of the eutrophication problem, Lund (1974) emphasized that in many cases the problem was related to the inputs of P. To illustrate this, the work of Schindler (1974) on Canadian shield lakes was used. Numerous small lakes allowed *in vivo* studies of lake fertilization. Lake fertilization with C, N, and P caused a prolific bloom of *Anabaena spiroides*, whereas additions of C and N did not produce any increases in algal biomass. Lund (1974) and Schindler (1974) considered that generally, N would be the second most important nutrient relevant to the control of eutrophication. The precise relationships found between algal standing crops and total P measurements (Dillon and Rigler, 1974; Jones and Backman, 1976) reinforce the suggestion that P is the controlling nutrient in many lakes; this also includes lakes where low N:P ratios should favour N limitation (Dillon and Rigler, 1974). Another interesting

feature of the lake fertilization experiments (Schindler, 1977) was that on reducing the ratio of N:P in the applied fertilizer, N-fixing blue-green algae became dominant over the green algae which appeared at high N levels. Consequently, the fixation of N accounted for a large part of the N input and maintained the ratio of total N to total P at levels similar to those in the fully-fertilized lakes. This further stresses the importance of P as a growth-limiting nutrient, when N-fixing blue-green algae are present in a body of water.

Following on from these studies, Schindler (1977) concluded that the removal of N from inputs to many lakes may actually affect water quality adversely, by causing low N:P ratios which favour the vacuolate, N-fixing, blue-green algae, that are most objectionable from a water-quality standpoint. Conversely, when P control causes an increase in the N:P ratios, the less objectionable green algae dominate (Edmonson, 1972a). It appears unlikely that P limitation is as simple as some workers suggest and, in fact, only initial growth may be stimulated by P, after which other limitations may become important. Edmonson (1972b) suggested that an interaction between N and P may be of importance in that the effectiveness of a given P supply may depend on the N concentration.

Other nutrients have also been implicated, such as carbon (Lange, 1967; Sakamoto, 1971; Kerr et al., 1972) and certain trace elements (Goldman, 1972), as important growth-limiting nutrients. Inputs of P, however, have been shown to produce the greater number of positive algal-growth responses in studies to-date. More important is the fact that, unlike N and C, some degree of control of the levels of P entering waters is possible.

2.1.2 Importance of particulate-phase phosphorus

The methodology for assessing the P status and the potential bioproductivity of a water body is gradually changing from one of measuring the concentration of DIP and changes in algal standing stocks in waters, towards determining the contributions made by inputs and DIP in source materials to the supply of biologically-available P in waters. Although the actual concentrations of DIP immediately available for primary production may be very low, there is often a substantial reservoir of P in the form of particulate material carried by rivers and streams, and which may enter lakes. Particulate material may contribute up to 95% of the total P entering streams from agricultural land during storm periods (Timmons et al., 1968; Sharpley, 1977; Rennes, 1978). The importance of the contribution made by particulate P to biologically-available P, however, has been disputed. The ability of suspended sediment in rivers and of lake muds to reduce DIP concentrations to 0.01 mg P l^{-1} or less (Gesner, 1960; Latterell, 1971; Shukla et al., 1971), which is believed to be below the critical growth level for some algae (Chu, 1943; McGaughey et al., 1969), has been established. This led Latterell et al.(1971) to suggest that neither sediment nor eroded soils contribute significantly to lake eutrophication. Mackenthun et al. (1968), however, reported an algal bloom in waters containing no more than 0.01 mg P l^{-1} and levels of 0.001 mg P l^{-1} have been recorded after depletion by algal growth (Rodhe, 1948; Mackereth, 1953; Hutchinson, 1957; McColl, 1972).

Soil and sediment P could be very important sources of available P through the desorption of adsorbed P. The possibility that lake sediments may function as a reservoir of available P in eutrophic lake waters has

long been acknowledged (Mortimer, 1941; Lund, 1959). Goldman (1960) stimulated algal growth by the addition of a lake mud extract that was 19 times higher in N and 11 times higher in P than the lake water in which the growth took place. In a similar study, Gahler (1969) found that resuspended lake sediments provided large amounts of N and P for algal growth. Taking this a stage further, Porcella et al. (1969) observed that the extent of algal growth in microcosm studies was directly related to the amount of P in different sediments. It could be argued that the results of these studies are of limited application because sediments would not be in direct contact with the photic zone or the algae, except in very shallow lakes. It has been reported that lake sediments separated from algae by dialysis tubing failed to stimulate algal growth (Fitzgerald, 1970; Golterman, 1973a). Golterman (1973a) suggested that diffusion through the dialysis membrane was so slow that IP was resorbed by the mud. Growth occurred in the control flasks where mud-algae contact was established. Sagher and Harris (1972) have shown that muds from the same lakes as those studied by Fitzgerald (1970) supported algal growth when not enclosed by dialysis tubing. Whereas the study of lake sediments in laboratory cultures may provide questionable evidence of their P availability, because of the artificial conditions involved, Wilding et al. (1974, 1977) have observed decreases in total sediment P, N, and C in open lake experiments, corresponding to a period of exponential blue-green algal growth during late spring and early summer. There was also an indication of sediment OP mineralization. In Lake Houston, Texas, Abbot (1957) observed that although the lake supported phytoplankton growth, no dissolved P was detectable in the lake water. A study of colloidal clay particles, which were derived from runoff in the watershed and were

suspended in the lake, suggested that the planktonic algae derived their P from the colloidal material. A similar association between particulate material and bioproductivity has been reported by Goldman et al. (1973) who found that the extent and intensity of the sediment plume produced by the Upper Truckee river in Lake Tahoe, as determined by aerial photography, was highly correlated with biological productivity. Kluesner and Lee, (1974) estimated that approximatley 80% of the annual total P input into Lake Wingra, Madison, Wisconsin, was from urban runoff and 40% of that was particulate P associated with the fine solid fraction (<43 μm), which was only 5.9% of total solids. Bioassays carried out with Selenastrum capricornutum by Cowen and Lee (1976) indicated that the equivalent of 30% of the particulate P was available for algal growth. It has also been shown that clays extracted from topsoils are able to supply P for the growth of Lemna minor (Healy and McColl, 1974). More recently McColl (1975) used a Chlorella sp. in bioassays to investigate the amounts of "available" P in some New Zealand clays, soils, and lake sediments. The clay fraction, which may comprise a large percentage of the particulate material in rural runoff (Sharpley, 1977) was found to support the largest algal growth.

Together with the previous studies on lake muds, the above studies with suspended sediments and topsoil clays constitute good evidence that particulate-phase P, derived from rural and urban runoff, may play an important role in the accelerated eutrophication of fresh waters. This has particular importance for New Zealand, where superphosphate fertilizer is applied to grazed hill-country pastures, from which runoff and erosion are a common feature (Rennes, 1978). In this regard, Fish (1969)

considered that P resulting from the topdressing of agricultural land was the most important cause of eutrophication of lakes in the Rotorua area (New Zealand). If measures to control the inputs of particulate P to waterways are to be implemented, then the various source materials (i.e., particulate material in rural and urban runoff), and the biological-availability of P in these source materials requires investigation.

2.1.3 Transport of particulate-phase phosphorus in waters

Sediment transported in flowing waters is primarily derived from farm lands (Lee, 1970). A loss of 1mm of good topsoil from 1000 ha could result in 15 tonnes of P moving into water courses (Cooke and Williams, 1973). The amounts of particulate material entering streams and rivers are primarily dependent upon rainfall intensity, physical and chemical stability of the eroding soil, and the volume and energy of run-off waters. Also, erosion is a selective process with respect to particle size. The finer particles (e.g. clays), which are predominantly carried in runoff, also contain the highest amounts of P (Syers et al., 1969). Greater selectivity of fines, and consequently of particulate-phase P, will occur as the energy of surface runoff decreases (Ryden et al., 1973). Although the P content of surface runoff will increase as the energy decreases, this does not produce an increase in particulate P load, because total sediment loads decrease as well.

The particulate material carried in streams may be divided into bed load and suspended load. The bed load may contain resuspended stream-bed sediment which moves close to the stream floor, whereas the suspended load, consisting of clay- and silt-sized particles, moves at approximately the same velocity as the water and, therefore, as dissolved P forms (Johnson and Moldenhauer, 1970). Therefore, it would appear that

suspended load, containing a high proportion of finer materials enriched in IP and OP, could be by far the most important potential contributor of biologically-available particulate P carried in streams. Not only does flowing water preferentially select the P-rich fine material, but differential sedimentation in a lake or a slow-flowing part of a stream or river will ensure that the finer material is deposited last, i.e., at the sediment-water interface, where it may best be used as a potential source of biologically- available P.

2.2 Particulate Phase Phosphorus

2.2.1 Origin and forms

2.2.1.1 Inorganic

Phosphorus is the eleventh most abundant element in igneous rocks and occurs in a large number of different minerals, of which the apatites ($Ca_{10}(PO_4)_6(OH, F, Cl, \frac{1}{2}CO_3)$) are by far the most abundant and important species (van Wazer, 1961). Volcanic rocks usually have higher P concentrations than igneous rocks. All secondary forms of P are initially derived by weathering, their ultimate destination being the oceans. Sedimentation of P adsorbed by aragonite ($CaCO_3$) and present within biological detritus has produced sedimentary rocks rich in P. With the progress of time, these rocks have been uplifted and the weathering process has continued.

In soils, particulate IP may conveniently be divided into primary and secondary forms. Primary IP refers to that in parent materials, such as apatite and the less common mineral monazite, whereas secondary IP includes IP which is adsorbed (non-occluded) and absorbed (occluded) by soil components. Until recently, discrete Fe and Al phosphates were

considered to be important forms of secondary IP in non-calcareous soils (Cole and Jackson, 1952; Kittrick and Jackson, 1956; Huffman, 1962). It is generally now accepted that these forms may only be important in highly-fertilized soils (Huffman, 1969; Syers and Williams, 1978) and that secondary IP exists in non-calcareous soils primarily as IP adsorbed and absorbed by short-range order (amorphous) components such as oxides and hydrous oxides of Fe and Al (Saunders, 1965; Syers et al., 1971; Ryden et al., 1973) and aluminosilicates (allophane, Cloos et al., 1968). Oxides and hydrous oxides are very common in soils (Hsu, 1964), their relative amounts depending on parent material composition, climate, and soil drainage conditions. Also, they occur mainly as surface coatings on other soil components. For example, Roth et al. (1969) have reported their presence on clay mineral surfaces. This large surface area exposes many -OH and $-OH_2+$ groups which can undergo ligand-exchange reactions with P ions (chemisorption) and usually gives rise to a high P sorption maxima. Therefore, clay fractions already high in P can further be enriched by the sorption of added fertilizer IP and of organic P esters.

Several studies have shown that the sorption of IP is an initially rapid process, which is followed by a much slower reaction which probably results from the diffusion of IP into the amorphous oxides and hydrous oxides of Fe and Al (McLaughlin et al., 1977; Ryden et al., 1977b). These amorphous components are believed to retain soil IP in two main forms, namely, chemisorbed IP and weakly-sorbed IP which results from a potential-determining sorption of a more-physical nature (referred to as more-physical sorption by Ryden et al., 1977a). The weakly-sorbed IP is reversibly held with respect to solution IP concentration. As expected,

the amounts of weakly-sorbed IP are considerably higher in fertilized soils and in topsoils compared to subsoils (Ryden and Syers, 1977b).

Because finer-fractions, particularly clay- and fine silt-sized materials, represent by far the major particulate P carried in streams (Scarseth and Chandler, 1938; Ryden et al., 1973), the above mentioned forms of sorbed IP may play an important role in determining the amounts of biologically-available IP in streams.

Apatite may only be important in streams draining weakly-weathered soils (Williams et al. 1969). The low solubility of apatite in water (0.03 mg l^{-1}, at pH 7; Ryden et al., 1973) may limit its biological importance. In fertilized, neutral or calcareous soils, more soluble calcic fertilizer reaction products may provide an important source of available P entering streams from runoff.

Because IP absorbed by amorphous soil components may be expected to show little biological availability and because apatite is relatively insoluble, it would appear that in any assessment of the biological availability of particulate-phase IP attention should be directed to the study of IP which is adsorbed by soil components.

2.2.1.2 Organic

Difficulties encountered in the extraction in an unaltered form, of organic P (OP) from soils, have caused problems with the identification of such compounds. In the past, the convenient split between alkali-soluble humic compounds and acid-soluble fulvic compounds has been used as the main criterion for characterizing soil OP. The known OP constituents of soil have been reviewed by Anderson (1967). Many New Zealand grassland soils contain relatively large amounts of OP; it is well established that OP levels increase as pasture development proceeds (De Turk, 1938;

Jackman, 1955; Walker, 1956; Williams and Donald, 1957; Walker and Adams, 1958). In the New Zealand soils studied by Walker and Adams (1958), OP constituted 74% of the topsoil P. Anderson (1964) has shown that approximately 50% of the total P in Scottish agricultural soils occurs in the organic form. Only approximately 50% of this soil OP has been identified, however, owing to the complex nature of OP associations with the humic and fulvic acid complexes (Thomas and Bowman, 1966; Moyer and Thomas, 1970; Osomoto and Wild, 1970; Schnitzer and Khan, 1972). These polydispersed mixtures of higher molecular weight, structure less polyanions, which are mainly but not exclusively formed by reactions of phenolic and nitrogenous compounds, are known to form complex linkages with metals (Lévesque and Schnitzer, 1967a, 1967b; Lévesque, 1969; Haworth, 1971). Such complexes are believed to retain IP and OP by sorption and ion-exchange reactions, as well as by bonding which is stronger than that in P ester and which are not subject to alkaline hydrolysis (Anderson and Malcolm, 1974).

The major forms of OP identified in soils have been inositol phosphates (Anderson, 1967; Cosgrove, 1967; McKercher, 1968), nucleic acids (Anderson, 1961), and other phosphate esters, such as phospholipids (Baker, 1977), sugar phosphates, and phosphoproteins (McKercher, 1968). The forms added to soil in the greatest amounts from plant and animal material are probably the nucleic acids and the simple sugar phosphates. These are quickly mineralized, however, or otherwise utilized by soil microorganisms. Although added in small quantities, other esters, such as inositol hexaphosphates, are stabilized in soils to the extent that they may accumulate to form a large part of the OP present. Their accumulation, despite the presence of soil phytase, has been attributed to the formation

of insoluble Fe and Al salts in acid soils and insoluble Ca salts in calcareous soils (Jackman and Black, 1951) and to their adsorption by soil components which inhibits hydrolytic enzymes (Greaves and Webley, 1969).

Inositol polyphosphates may consitute up to 50% of the OP in soils (Anderson, 1967); this value varies however from 0 to 62% when a wide range of soils is considered (McKercher and Anderson, 1968; Williams and Anderson, 1968; Martin, 1970; Osomoto and Wild, 1970). The sources of inositol polyphosphates are plants, where it is the chief storage form of both inositol and P in seeds, and animal tissue (L'Annuziata, 1975). There is no information as to the quantities of soil inositol polyphosphates which are synthesized by soil microorganisms or derived from decomposing tissue. The larger part of soil inositol polyphosphates consists of the hexaphosphates (phytate), present as myo-, chiro-, and scyllo-inositol isomers, with myo-inositol hexaphosphate being the most abundant. The hydrolysis products mono, di, tri, tetra, and penta inositol phosphates are present only in minor quantities (Anderson, 1975). The scyllo-isomer is believed to be of microbial origin because it has not been found in animal or plant tissue (Cosgrove, 1966a).

The other P esters comprise only a small part of the soil OP, with nucleic acids accounting for 5 - 10% and phospholipids, sugar phosphates, and phosphoproteins less than 1 - 2% (McKercher, 1968).

In addition to associations with the humic and fulvic matter of soils (Anderson and Hance, 1963; Cosgrove, 1966b), OP can be sorbed through the phosphate group to the same Fe and Al oxides and hydrous oxides which sorb IP (Sommers et al., 1972; Anderson et al., 1974). Anderson and Arlidge (1962) have shown that the sorption of inositol phosphates increases with the number of phosphate groups in the molecule.

Thus sorption of OP is primarily controlled by interactions of the phosphate group. Treatment of a soil with oxalate to remove amorphous Fe and Al greatly reduces the capacity of the soil to sorb inositol hexaphosphate (Anderson and Arlidge, 1962). Pure clay minerals and preparations of amorphous Fe and Al hydrous oxides were also shown to sorb inositol polyphosphate, nucleic acids, and nucleotides strongly at low pH values (Goring and Bartholomew, 1952; Anderson and Arlidge, 1962). In fact, six acid soils in Scotland showed a preferential adsorption of inositol hexaphosphate over IP (Anderson et al., 1974). This probably results from the fact that the organic moiety of the P ester can also be sorbed by soil components. For example, nucleic acids and nucleotides protonated at pH < 5 can displace exchangeable cations on clay surfaces (Jordan, 1955). Physical sorption such as van der Waals forces, ion-dipole interactions, and the hydrophilic-hydrophobic nature of some of the organic molecules can contribute to the retention of larger molecula weight organics (Greenland, 1965).

As with IP, the greater percentage of OP in soils is associated with the clay-and silt-sized fractions (Williams and Saunders, 1956; McDonnell and Walsh, 1957; Svers et al., 1969). In a study of particulate P, clay- and silt-sized fractions appear to be by far the most important contributors to both organic and inorganic soil P.

2.2.2 Chemical characterization

2.2.2.1 Inorganic

The characterization of soil IP has been attempted by the use of a range of extraction procedures, often used in a sequence. The reagents were originally selected (Chang and Jackson, 1957) for their ability to solubilize the IP contained in minerals such as variscite,

strengite, and apatite. For example, the modified Chang and Jackson procedure used by Peterson and Corey (1966) uses the following sequence of reagents: 1M NH_4Cl to remove "easily-soluble P"; 0.5M NH_4F at pH 8.2 to remove Al-P; 0.1M NaOH to remove Fe-P; 0.3M sodium citrate and sodium dithionite buffered with sodium bicarbonate to remove reductant-soluble or occluded P (CDB-P); and 0.25M H_2SO_4 to remove Ca-P. More recent research, however, has shown that the reagents in this procedure and other modifications are not specific for individual IP compounds as first thought (Dalal, 1973a). There is considerable evidence that a large percentage of the IP associated with oxides and hydrous oxides of Fe can be removed by NH_4F, as well as any Al-P (Bromfield, 1967). The high pH (8.5) of the reagent may result in the desorption of IP sorbed by Fe components (McLaughlin et al., 1977). Extracted IP may also be resorbed during the NH_4F extraction by soil Fe components (Fife, 1962; Williams et al., 1967) and by CaF_2 formed in calcareous soils (Syers et al., 1972). This resorbed IP, excepting CaF_2-P, is released during the subsequent 0.1M NaOH extraction. The CDB-P fraction is thought to be derived from IP held within the matrices of crystalline iron oxides and hydrous oxides (Bauwin and Tyner, 1957) and IP absorbed by amorphous oxides and hydrous oxides of Fe (Ryden et al., 1977c). In calcareous soils, the CaF_2-IP formed during the NH_4F extraction appears as acid-extractable IP (Syers et al., 1972). Certain calcium-containing fertilizer reaction products may also appear in this fraction.

A similar type of chemical fractionation procedure has been used to characterize IP in lake sediments (Harter, 1968; Williams et al., 1971a, 1971b). These studies, along with those comparing exchangeable P levels in lake sediments (Li et al., 1972, 1973) and soils (Tandon and Kurtz, 1968;

Ryden et al., 1977b) with chemically-characterized forms of IP, have provided an improved understanding of the forms of particulate P in such materials. It is now believed, however, that chemically-characterized P fractions describe more the "mode of occurrence" of soil IP, rather than its chemical nature. For example, in a sequential fractionation scheme the IP adsorbed at the surface of hydrous metal oxides and allophane is extracted by NH_4F and NaOH reagents and this has been termed non-occluded P (Syers and Walker, 1969). The P retained within or absorbed by amorphous and crystalline oxides and hydrous oxides of Fe and Al has been termed occluded P and is extracted by a citrate-dithionite-bicarbonate reagent. Acid-extractable P includes discrete phase IP, such as apatite and calcic fertilizer-soil reaction products found in highly-fertilized soils.

Several other chemical extraction procedures have been used as single-solution tests to extract soil IP in an attempt to characterize the amount of plant-available P in soils (Truog, 1930; Bray and Kurtz, 1945; Olsen et al., 1954; Ryden and Syers, 1977b). With the exception of the procedure developed by Ryden and Syers (1977b), each of these procedures extracts either a mixture of the P forms described above, or parts of those forms. Some of these procedures, including that for exchangeable P, will be discussed in Section 2.2.3.

2.2.2.2 Organic

Chemical characterization of OP has been limited by the inability to extract much of the soil OP in an unaltered form. This problem is also compounded by the extremely complex nature of soil OP. Early workers focussed their attention on the extraction and measurement of total soil OP (Mehta et al., 1954; Saunders and Williams, 1955; Walker and Adams,

1958) and not on the identification of specific OP compounds. An extraction procedure which involves an acid pretreatment followed by a cold and then a hot alkali extraction (Mehta et al., 1954), has been adopted, with minor modifications, as the standard method of extracting OP from soils (Anderson 1960; Dormaar and Webster, 1963; Martin, 1964a).

Other workers have experimented with reagents which are milder than the strong alkalis, in attempts to extract OP compounds intact. Boswall and de Long (1959) extracted 75 to 88% of the total OP from two soils with 8-hydroxyquinoline in benzene, whereas MacLean (1965) used a single treatment with $NaHCO_3$, at pH 10, and found that 98% of the OP removed by the Mehta extraction procedure was extracted. With a view to chemical analysis of intact OP in the extract, Halstead et al. (1966) used acetylacetone and sonication of the extractant-soil mixture to solubilize OP. The use of this milder extraction procedure was successful in reducing the degree of hydrolysis of the extracted OP compounds.

Because the major part of soil OP is extracted by alkali (Halstead and Anderson, 1970; Anderson and Malcolm, 1974), a considerable number of investigations into the nature of soil OP have been concerned with the fractionation of alkali extracts by either ion-exchange chromatography (Cosgrove, 1963; Martin, 1964a; Martin and Wicken, 1966; Halstead and Anderson, 1970; Martin, 1970; Anderson and Malcolm, 1974) or, more recently by gel chromatography (Schnitzer and Skinner, 1968; Moyer and Thomas, 1970; Steward and Tate, 1971; Swift and Posner, 1971, 1972). Using these procedures it has been found that alkaline soil extracts contain mono-phosphorylated carboxylic acids; inositol polyphosphates, nucleoside phosphates, glycerophosphates, and several unidentified esters (Martin, 1964b; Anderson and Malcolm, 1974), as well as forms of P which remain in

complex associations with humic acids (Anderson, 1961; Martin, 1970; Martin and Molloy, 1971; Swift and Posner, 1972). Most of the work involving ion-exchange fractionation of soil extracts has been directed specifically towards the identification of inositol polyphosphates, the charged phosphate groups of which interact readily with the resins. Ion-exchange resins, however, are not suited to the study of more complex phosphates. The technique of gel chromatography appears to be more promising for the more complex soil organic material, allowing the fractionation and identification of OP according to molecular size. These two procedures and their use in characterizing particulate OP are discussed further in Section 9.1.

A rather novel method for characterizing soluble OP in lake water was used by Herbes et al. (1975). The presence of inositol hexaphosphate in a high and in a low molecular weight fraction was determined by comparing the enzyme kinetics involved in the release of IP, by wheat-bran phytase, from the lake-OP fractions and from pure inositol hexaphosphate. The hydrolyzable OP, assumed to be inositol hexaphosphate, was present in equal quantities in both the high and low molecular weight fractions.

Although the chemistry of soil IP is reasonably well understood and well documented, the chemistry and biochemistry of soil OP are much less well established. The exceptions to this are the simpler sugar phosphates, nucleic acids, and other P esters which are able to occur free or in less close association with more complex organic materials.

2.2.3 Assessment of biological availability

2.2.3.1 Inorganic

Several methods have been used to assess, directly and indirectly, the biological availability of particulate IP in soils and sediments. These

are briefly reviewed in this Section.

2.2.3.1.1 Sorption and desorption. Orthophosphate ions in solution (DIP) are clearly the most directly available form of P in waters. The ability of a particulate P source to sustain a given level of DIP is a major factor determining the total quantity of P which is potentially available for aquatic plant growth. The ability of eroding soil material to sorb DIP in streams is also important (Wang and Brabec, 1969).

Several workers have used the data gained from IP sorption studies to give an indication of the likely availability of particulate IP in both eroded soils (Taylor, 1967; Taylor and Kunishi, 1971; Ryden et al., 1972a, 1972b) and lake sediments (Williams et al., 1970; Shukla et al., 1971). As pointed out by Ryden et al. (1972a), unless soil:solution ratios and P additions realistic in terms of the stream environment are adhered to, the data obtained are of limited value. Sharpley (1977) has also stressed the importance of selecting only the particle size fraction which is transported, when studying the sorption characteristics of suspended stream sediment source materials. In the work reported by Sharpley (1977) the < 30-μm fraction accounted for 90% of the particulate material in surface runoff from pasture and this released eight times the amount of IP released by the same weight of whole surface soil (0 - 5cm).

Both eroding-surface soil and stream-bank material have been implicated as materials which influence DIP concentrations in streams (Kunishi et al., 1972; Ryden et al., 1973; Gburek and Heald, 1974). Sharpley (1977) reported that although eroded topsoil (surface-runoff material) released IP to solution, the resulting DIP concentration could be decreased on contact with stream-bank material, which had a higher P-sorbing capacity. Similar

observations have been made by Taylor and Kunishi (1971), Kunishi et al. (1972) and Ryden et al. (1972) for whole soil and source materials. Kunishi et al. (1972) showed that during periods of high and low flow, the relative proportions of surface-runoff and stream-bank material, making up the suspended load could be used to describe the concentration of DIP present.

Although sorption-desorption studies may be used to provide estimates of DIP concentrations in runoff and streams, they do not provide an absolute value for the amount of biologically-available P. The data however, do provide an estimate of the likely amount of immediately-available IP; this is an important parameter in the initiation of algal blooms (Vollenweider, 1968). Because algal blooms can occur in the absence of detectable levels of DIP (Abbot, 1957; McColl, 1972) or, over the period of growth, may reduce DIP concentrations to undetectable levels (Thomas, 1973; Wood and Gibson, 1973) it is obvious that biologically-available P constitutes a fraction which is larger than readily-desorbable IP. Furthermore, the free energy of sorption for P ions and soil components may be such that a varying amount of exchangeable IP may remain on the soil, with essentially undetectable levels of IP appearing in solution. Because surface-sorbed IP can be extracted by NH_4F and NaOH it seems likely that a certain proportion of NH_4F- and NaOH-extractable IP may have a high availability for aquatic plant growth.

2.2.3.1.2 Isotopic exchangeability. In attempts to quantify the level of exchangeable particulate P and to determine its influence on the P status of surrounding water, use has been made of ^{32}P in isotope dilution studies. Work relevant to the influence of exchangeable P on freshwater DIP concentrations has been confined to the study of estuarine

and lake sediments (Hayes and Philips, 1958; Pomeroy et al., 1965; Li et al., 1972, 1973). Li et al. (1973) demonstrated that the levels of exchangeable P were consistent with the levels of NaOH-IP measured in the lake sediments studied by Williams et al. (1970). In similar work with soils, NaOH-IP was found to have a high degree of exchangeability (Dunbar and Baker, 1965). Tandon and Kurtz (1968) found that most of the isotopically-exchangeable P in widely-different soils was in the fractions extracted by NH_4F and NaOH, which were assumed to be Al-P and Fe-P, respectively, (see Section 2.2.2.1). The "Fe-P" fraction was found to exchange more slowly with ^{32}P than the "Al-P" fraction. Thus it can be assumed that in terms of relative exchangeability, the "Al-P" fraction is more loosely bound and therefore has a greater potential biological availability than the "Fe-P" fraction. Reductant-soluble IP and acid-extractable IP show little exchangeability (Chu and Chang, 1966) and therefore may be considered to be more inert.

2.2.3.1.3 Bioassay procedures. The most promising technique for measuring biologically-available particulate P is that of the bioassay. In this procedure, all other nutrients and conditions are favourable for growth, and the rate of production or final biomass values are related simply to the concentration of available P. The bioassay format (Section 2.3) is usually similar to the Provisional Algal Assay Procedure (PAAP, Environmental Protection Agency, 1969). The particulate P material is introduced as the only source of P in an algal culture flask, and the resultant growth is related to growth in solutions of known DIP concentration. Chiou and Boyd (1974) studied muds made from twelve types of soils as the only source of P in cultures of Scenedesmus dimorphus. The muds supported a range of algal growth, from no growth to

as much as that maintained by 0.5 mg l^{-1} of DIP. Algal growth was then correlated with the amounts of soils IP which were extracted by: (1) the P free nutrient media used; (2) 0.05N HCl + 0.025N H_2SO_4; (3) 0.002N H_2SO_4 + 3g K_2SO_4 l^{-1}; and (4) 0.1N HCl + 0.3N NH_4F. All correlations were significant at the 1% level of probability, but better correlations were obtained with reagents (3) and (4), than with (1) and (2). In a similar study, McColl (1975) investigated the ability of New Zealand topsoil clays, soils, and lake sediments to supply P for the growth of Chlorella vulgaris. The amount of P considered to have been supplied by the clay, when chlorophyll production was compared to algal growth on DIP, was estimated to be higher than water-soluble IP or Truog-IP and closer to 0.5M H_2SO_4-extractable IP. McColl then estimated that an amount of P equivalent to between 24 and 81% of the IP extracted by 0.5M H_2SO_4 was depleted from topsoil clays; approximately 25% from lake sediment; and 0.3 to 1.0% from subsoils. Cowen and Lee (1976) carried out bioassays with Selenastrum capricornutum (Environmental Protection Agency, Algal Assay Procedure) on the fine-particulate fraction (<43 µm) of urban runoff and found that equivalent to 30% of the particulate P was available for algal growth. On comparing the estimates of biologically-available P, with operationally-defined forms of extracted particulate IP, namely, acid (8.1ml conc. HCl + 1.3ml conc. H_2SO_4 21^{-1}), base (0.1M NaOH + NaCl), and resin (20 - 30 mesh Dowex 1 x 8 anion-exchange resin), Cowen and Lee (1976) reported that the estimates were closer to the amount of IP extracted by the alkali and the resin than by the acid. In the majority of samples, biologically-available P was higher than that measured by the alkali. Although, the above studies demonstrate clearly that soils and muds are able to supply P for algal growth, and that

estimates of available P can be made from equivalent algal growth on DIP, they do not provide information as to which chemical or physical forms of particulate P are biologically available. Also, by correlating the estimated amounts of biologically-available particulate P to the amounts of extractable particulate IP only, these studies have not considered the potential biological availability of particulate OP.

In an attempt to identify forms of biologically available particulate IP, Golterman et al. (1969) bioassayed Fe and Ca phosphates using Scenedesmus obliquus. The Fe-P was utilized completely in a medium containing sufficient $NaHCO_3$ to maintain the pH at 8; under the same conditions, 30% of the P in hydroxyapatite was also depleted. Subsequently, the same workers, investigated the availability of "Fe-P" and "Ca-P" in Dutch lake muds. From the chemical fractionation of the IP in the muds before and after algal growth, and after correcting for algal P contribution (see Section 4) to those fractions, it was found that 8 to 44% of the 0.1N NaOH-plus 0.5N H_2SO_4 -extractable IP was depleted. These fractions were assumed to be Fe- and Ca-bound, respectively, even though IP added as Fe and Ca phosphates could not be recovered quantitatively from the sediments by these reagents. From a similar study on Wisconsin lake sediments, Sagher and Harris (1972) concluded that a substantial proportion of the IP, in a range of calcareous and non-calcareous sediments, could be utilized by algae. Again, the most available form of IP was shown to be the 0.1N NaOH fraction. In contrast to the work of Golterman et al. (1969), Ca-P IP (acid extractable) had a very limited availability. In more recent studies, Golterman (1973b) has discarded the more classical extractants for nitrloacetic acid (NTA). A 0.01N NTA solution extracted amounts of IP from sediments which were equivalent to those "bound by Fe

and Ca". Using the Ca-NTA salt, it was possible to selectively extract Fe-P. This has the advantage over NaOH or H_2SO_4 in that the sediment residue can be added to algal cultures as the sole P source. The amount of NTA-extractable IP was similar to the amount depleted by algal growth. Also after NTA treatment, a sediment was not able to support algal growth. This apparent complete availability of "P bound by Fe and Ca" partly contradicts the earlier work published by Golterman and co-workers (Golterman et al., 1969) and that of Sagher and Harris (1972). In a study of the P status of eutrophic lake-bottom sediments, Wildung et al. (1977) have related the reduction in the non-occluded IP fraction of sediment to biological production and demand for P in surface waters. By employing the serial extraction technique of Chang and Jackson (1957), as modified by Williams et al. (1967), sediment IP changes were found to be primarily due to reductions in the quantity of IP extracted by NaOH and citrate-dithionite. Wildung et al. (1977) hypothesized that these fractions may serve to define biologically-available P. This work partly complements the findings of Golterman et al. (1969), Sagher and Harris (1972), and Golterman (1973b). In a previous study of the same lake-bottom sediments Wildung et al. (1974) showed that decreases in total sediment OP occurred simultaneously to the decreases in sediment IP which they had related to demand for P in surface waters. In their later study (Wildung et al., 1977) and in the study of Golterman et al., (1969), however, the availability of sediment OP, which was removed in the extractants used to monitor sediment IP, was not considered. Residual sediment OP, which remained after the extraction of sediment IP, was measured, but as expected this form did not appear to be depleted by biological activity.

If a bioassay is combined with an appropriate particulate IP

fractionation scheme, a more meaningful and quantitative determination of the relative availability of particulate IP forms can potentially be achieved. Providing that problems inherent in IP fractionation schemes are recognised, i.e., the reagents used are not specific for distinct chemical forms of particulate IP, useful interpretations may be drawn from the data obtained. Because of the impossibility of being able to completely separate algae or bacteria grown on particulate P, methods of correcting for the algal contribution to the chemically-defined P fractions are required. It is envisaged that if the amount of sediment OP also removed by the extractants used for particulate IP is also measured, this may provide valuable information as to the biological availability of sediment OP.

2.2.3.2 Organic

As discussed in the previous Section the results obtained by Wildung et al. (1974) suggest that lake-bottom sediment OP may be depleted during periods of biological productivity in the surface waters. There does not appear to be any published information, however, concerning the chemical characterization of biologically-available particulate OP.

The desorption of sorbed OP from soil has not been studied extensively and in fact Hanapel et al. (1964) reported that most of the OP leached from soils was present in a particulate form. Considerable evidence is available to suggest that particulate OP can be utilized for growth. It has been shown that soluble alkaline phosphatase enzymes released large amounts of DIP from organic material suspended in Lake Kinnerett, in northern Israel (Berman, 1969); the release was associated with a heavy algal bloom. Cooke and Williams (1973) also suggested that eroded or illuviated soil OP may be hydrolyzed in a similar manner. Such

phosphatase enzymes, which act extracellularly (Blum, 1965), are capable of hydrolyzing several organic and polyphosphate compounds (Clesceri and Lee, 1965; Davis and Wilcomb, 1967; Reichardt, 1971). Organic P esters such as inositol hexaphosphate and nucleic acids, have been shown to act as P sources for terrestrial plants grown in sand or water (Whiting and Heck, 1926; Rogers et al., 1940; Martin, 1973). The mineralization of several OP compounds in soil has been reviewed by Cosgrove (1967) and it can probably be assumed that similar reactions would occur in lake sediments and suspended materials. The factors governing the rate and extent of sediment OP mineralization, however, have yet to be defined. Obviously, of major importance are the extracellular phosphatases and phytases which are produced prolifically by numerous plants, algae, and bacteria when DIP concentrations are very low. Under such conditions, algae have been shown to use inositol polyphosphates, glycerophophates (Chu, 1946), nucleoside P (Provasoli and McLaughlin, 1963), phospholipid (Miyachi et al., 1965) and a variety of P-based detergents (Fosberg et al., 1967; Stewart and Alexander, 1971). Herbes et al. (1975) showed that 50% of the soluble OP in lake water was hydrolyzable by phytase, and showed similar kinetics to the hydrolysis of myo-inositol hexaphosphate. The hydrolyzable OP was present in both high and low molecular weight fractions, separated on Bio-Gel P60. It was proposed that the low molecular weight fraction was free inositol polyphosphate and the high molecular weight fraction may have been composed of inositol polyphosphates bound to proteins, lipids, or fulvic acids. It has been shown, however, that OP compounds are less readily hydrolyzed when they are associated with fine sediment (Phillips, 1964; Greaves and Webley, 1969; Rodel et al., 1977). Greaves and Webley (1969) suggested that the decrease

in the hydrolysis of sediment-associated myo-inositol hexaphosphate was caused by the adsorption of both the substrate and the enzyme by clay minerals. Other workers have shown an opposite effect, where concentration of proteolytic enzymes on kaolinite (Estermann and McLaren, 1959) and on a cationic form of montmorillonite (Aomine and Kobayashi, 1964) appeared to increase hydrolysis.

Although there appears to be substantial evidence to suggest that both dissolved and surface-sorbed OP can be hydrolyzed in the aquatic environment, the amounts and forms of available OP in particulates, and the mechanisms by which they can be made available for plant growth, remain unclear.

2.3 Bioassays, as Analytical Tools

Rodhe (1969) has discussed the historical use of nutrient concentrations and rate of organic matter production as criteria for classifying the trophic status of lakes. Considering eutrophication as a result of nutrient enrichment, with low external carbon inputs, the photsynthetic potential of the water becomes important. Consequently, in order to measure the trophic state of a water body, it is necessary to measure aquatic algal or plant production.

2.3.1 Algal bioassays

Because algal growth is the most easily monitored form of primary production and is the major manifestation of a eutrophic water body, P and other nutrient availability studies have come to involve algal biomass production as an index of nutrient availability. Algal bioassays are capable of providing far more sensitive results for nutrient concentrations

than chemical analyses, as well as providing a direct measurement of biologically-available nutrients. Bioassays usually involve the addition of a selected alga to a nutrient sample; nutrient availability can be determined from the algal biomass produced and the nutritional status of the algae (PAAP, Environmental Protection Agency, 1969; Fitzgerald, 1972; Healey, 1973). Although other techniques have been used for the bioassay of water samples (Goldman, 1964; Wetzel, 1965; Chamberlain and Shapiro, 1969; Fitzgerald, 1972; Cain and Trainor, 1973) the bioassay of nutrient source materials, such as particulate material, requires that the algae are treated as an analytical extractant and, as such, must be standardized. Also, the algal parameters used to indicate nutrient availability should be standardized. When standardizing a bioassay procedure it is important to consider the following: (1) the test-species must be able to dominate all algae indigenous to the sample, if non-sterile samples are to be used; (2) bloom-forming, blue-green algae are particularly suitable for P bioassays. Fitzgerald (1972) has suggested that test algae should be selected on the basis of the alga which dominates the water body to be studied, or which dominates the water body into which the nutrient source materials will pass, because not all algae will respond to the same enviornment; (3) pre-starvation of the inocula is very important because nutrient stores within the algae may be larger than the concentration of nutrients in the sample (Gerloff and Skoog, 1954). Only pre-starvation can reduce the cell nutrients to insignificant quantities (Azad and Borchardt, 1970) and ensure that all algal growth responses are the result of nutrients in the sample; (4) the physical conditions of the bioassay, such as light intensity, temperature, CO_2 supply, and mixing can be varied in order to measure

either the nutrients available under conditions similar to the natural environment, or the maximum amount of available nutrient when optimal conditions for algal growth are used.

Obviously, sample preparation is important and there are arguments for sterilizing samples (Cain and Trainor, 1973) in order to standardize bioassays, and arguments against because sterilization considerably affects nutrient availability within the sample (Filip and Middlebrooks, 1975). The stimulation of bacterial growth during a bioassay can be a problem but this may be limited by the use of an N-fixing test algae and the omission of added nitrate. Bacterial growth only then becomes a problem in the latter stages of growth when nitrogenous, extracellular products from the algae may stimulate heterotrophic growth (Fogg, 1962; Watt, 1969).

The measurement of the algal mass produced by a nutrient in a bioassay is subject to problems because algal biomass cannot be measured directly. Thus, other cell parameters which appear to be closely related to cell growth can be used (Vollenweider, 1969b). These include packed cell volume (Kylin, 1964), dry weight (Fitzgerald, 1966; Reichardt, 1971), and turbidity at a set wavelength (Parr and Smith, 1976). These methods, however, cannot be used during the bioassay of particulates. Other methods for the measurement of algal biomass in the presence of particulates include cell counts (Golterman et al., 1969; Cain and Trainor, 1973; Javornicky et al., 1973; Sagher, 1974) which may be made using an automatic cell counter (Chiou and Boyd, 1974) if a non-filamentous alga is used, chlorophyll concentration of an extract (McColl, 1975; Golterman, 1976; Jones and Bachman, 1976; Paerl et al., 1976), and estimation of live biomass using analysis of adenosine triphosphate (ATP) concentration

(Holm-Hansen and Paerl, 1972; Paerl et al., 1976). In xenic cultures, ATP measurement would estimate both bacterial and algal biomass. Of the above methods, the determination of chlorophyll concentration in laboratory bioassays is perhaps the easiest to perform, and has the added advantage of being independently related to nutrient availability (Healey, 1973; McColl, 1975). The interference from other pigments only becomes a problem when the amount of dying or decomposing cells in a culture becomes high. For estimation of biomass, the determination of chlorophyll concentrations in extracts by measuring the absorbance of chlorophyll *a* at 663-665nm appears to be a reasonably accurate method (Talling and Driver, 1963).

Further information on nutrient availability during a bioassay may also be obtained by the measurement of changes in algal metabolism or cellular nutrient contents (Fitzgerald and Nelson, 1966). Healey (1973) has intensively studied these aspects for the symptoms of P deficiency in the blue-green alga *Anabaena variablis*. The possible use of such changes as loss of heterocysts, increasing alkaline phosphatase activity, increasing P-debt, and decline in chlorophyll *a*, protein, RNA, and cellular P as indicators of P deficiency have been considered. In a bioassay of particulate material, where *Anabaena* and the particulate material cannot be separated after algal growth yet the measurement of the depletion of specific particulate P fractions is desired, some of these algal cell changes could provide a useful method of comparing the P status of *Anabaena*, grown on particulate material, with the P status of *Anabaena* in P growth-control flasks. *Anabaena* of similar P status to those grown on the particulate material could then be selected for P fractionation studies. This may provide a more accurate determination of algal P contribution to

soil P fractions, than assuming that algal P content is constant throughout growth (Golterman et al., 1969) or constant between algae that have been exposed to different degrees of P availability. The bioassay of soil material precludes the use of boiling water-extractable P (Fitzgerald and Nelson, 1966) and increasing P debt as measures of algal P status, because of P sorption by the soil. Of the other measurements, chlorophyll *a* concentration and alkaline phosphatase activity can be performed on small aliquots taken from the bioassay. Also, they are simple and rapid determinations which could easily be accommodated in a routine analysis programme. Alkaline phosphatase activity measurement has the advantage of using a simple reaction which is mediated by a rather stable enzyme (half life 3 days at 18^{o}C; Reichardt et al., 1967). An increase in alkaline phosphatase activity appears to be specific to P limitation (chlorophyll *a* is not) in several algae (Kuenzler and Perras, 1965; Heath and Cooke, 1975). Enzyme levels in *Anabaena variablis* (Healey, 1973) increased as P was depleted and before growth rate began to fall; this indicates that alkaline phosphatase was controlled by the concentration of a P compound and was not controlled by growth rate. It also suggests that specific enzyme activity is directly related to available P concentrations in situations where no other nutrients are limiting. One disadvantage is that alkaline phsophatase activity does not respond to sudden additions of P, e.g., the inoculation of P-starved algae into a P solution, and the decrease in enzyme activity is gradual by the process of cell dilution. Sudden increases in P would not normally occur during a bioassay, so this is not a real problem.

As an estimate of biomass, chlorophyll *a* concentration is susceptible to variations in nutrient availability (Healey, 1973). During a bioassay

for P however, when other nutrients are adequately supplied, it may be an advantage that chlorophyll a concentration is independently related to P availability, as well as being a measure of biomass. If alkaline phosphatase activity is monitored simultaneously with chlorophyll a concentration during a bioassay, an increase in enzyme activity would confirm that a decline in chlorophyll a concentration was the result of reduced P availability.

From the literature reviewed it may be concluded that for bioassays of particulate-phase P using Anabaena, the monitoring of chlorophyll a concentration and alkaline phosphatase activity should give relatively accurate determinations of algal biomass and algal P status, respectively. Further evaluation of the two parameters, under the conditions involved in a bioassay of particulate P require investigation.

2.4 Conclusions

Inputs to waters which result in increased available P concentrations are, in general, the major accelerants of eutrophication. Particulate P source materials may play such a role. The nature of particulate IP is relatively well established, whereas the nature of much of the particulate OP fraction remains largely obscure. There is considerable evidence for the varying availability of particulate IP and OP for algal and bacterial growth. It appears, however, that no bioassay study to date has measured the amounts and forms of particulate IP and OP which are present in stream sediment source materials and are available for the growth of algae in fresh waters.

Only by study of the biological availability of particulate IP and OP is it possible to determine the potential impact of sediment loads on water

quality and, in turn, evaluate the importance of corrective measures to reduce such inputs.

SECTION 3

GENERAL METHODOLOGY

3.1 Algal Culture Procedures

3.1.1 Bioassay organism

The planktonic, blue-green alga Anabaena subcylindrica, isolated and described by Ms. Catherine Lam (Botany Department, Auckland University), was chosen for the study for the following reasons:

(1) It is frequently found as a dominant bloom-forming species in New Zealand waters.

(2) It has been found to be an ideal organism with which to work, because it is planktonic and gives a homogeneous culture under laboratory conditions.

(3) It is able to fix nitrogen (N), avoiding the need for an N source which may stimulate the growth of indigenous bacteria and algae.

(4) It has the ability to rapidly assimilate solution phosphorus (P), thereby providing optimum conditions for the depletion of a P source.

(5) Stock cultures can easily and inexpensively be maintained on agar slopes.

3.1.2 Media

Stock agar slope culture (Gorham's ASM-1, N- media (Gorham et al., 1964) plus 1% Davis Bacterial agar) were maintained under peiodic lighting (2000 Lux, cool, white fluorescent) at 15^{o}C. New slopes were prepared every 6 weeks.

Liquid cultures (Gorham's ASM-1, N- media) were initially adjusted to pH 7, and maintained under continuous lighting (4000 Lux, cool, white

fluorescent) at 28°C on a reciprocating shaker (90 r.p.m.).

3.1.3 Preparation of inocula

Experimental inocula were prepared in the following manner: A 2-litre Erlenmeyer flask containing 1-litre of P+ (as 0.139g l^{-1} of K_2HPO_4) media (ASM-1, N-) was inoculated with algae from the stock agar slopes. Over a period of 2 weeks the algae were grown to a known chlorophyll concentration, harvested by centrifugation at 6089 x G (Sorvall Superspeed RC-2B) for 10 min, and washed and resuspended in ASM-1, P-, N- media for 10 days of P starvation under liquid-culture growth conditions. Algal alkaline phosphatase activity was monitored during P starvation. When the appropriate level of P starvation was attained the cells were harvested again by centrifugation and resuspended in P- (ASM-1, N-) media to give an approximate cell concentration of 15mg ml^{-1}. A sample (10 ml) of this suspension was used to inoculate each experimental flask. During the later stages of experimentation, it was found that when 1 litre of P+ media (ASM-1, N-, 0.8mg P l^{-1}) was inoculated with algae from stock slopes, sufficiently P-starved algae were produced after 3 weeks growth under liquid culture conditions. Aliquots from this culture were used to inoculate the experimental flasks.

3.1.4 Bioassay technique

The growth media (ASM-1, P-, N-) had a negligible influence on particulate P availability (See Appendix Table I).

Samples (1g) of the < 30-μm material of various soils and stream-sediment source material were suspended in 1 litre of growth media (ASM-1, P- N-), which was adjusted to pH 7 over a period of 3 days. Each system was duplicated and a non-inoculated, mercuric-chloride poisoned, control flask for each material was incubated over the algal

growth period to ascertain whether there was any non-biological transfer of P between the different P fractions. All flasks were incubated overnight at 28°C before being inoculated with P-starved algae.

The particulate P systems and the algal-growth control systems (see Section 4) were then incubated until algal growth became P limited; denoted by a decrease in chlorophyll pigment levels and confirmed by the addition of 500 µg P to re-establish algal growth. Intermittently over the period of incubation, algal chlorophyll, whole-cell alkaline phosphatase activity, and algal and soil P fractions were determined. The data from the algal-growth control flasks provided the necessary corrections for the algal contribution to the soil plus algal P fractions. This permitted the rate and the extent of depletion of each soil P fraction to be calculated.

3.1.5 Alkaline phosphatase activity

Whole cell, alkaline-phosphatase activity was determined as follows: 1ml of the algal culture was incubated with 0.5M tris-hydroxymethyl-methylamine (2ml) (pH 8.6) and 10^{-2}M 4-nitrophenyl disodium orthphosphate (2ml) in a 12-ml polycarbonate certrifuge tube at 30°C for 30 min. The cells were separated by centrifugation (2700 x G, for 30 sec) and the absorbance of the supernatant was measured against an incubated reagent blank at 410 nm on a Pye Unicam SP 1800B spectrophotometer. Alkaline phosphatase activity was expressed in relation to biomass as µmoles 4-nitrophenyl-disodium orthophosphate hydrolyzed hr^{-1} μg^{-1} chlorophyll (µmoles pNP hr^{-1} μg^{-1} chl).

3.1.6 Development and evaluation of biomass measurements

Several methods for estimating algal biomass were considered (Section 2). Because of the presence of soil material in the algal systems

and because a rapid method was required to fit into the routine analysis programme, only two methods were evaluated; namely (1) the measurement of organic carbon, as a standard related to dry weight and (2) the determination of chlorophyll concentration.

3.1.6.1 Organic carbon and dry weight

The acidified potassium dichromate digestion procedure of Walkley and Black (1934), as modified by Bremner and Jenkinson (1960) was used to oxidize the organic material present. When used for soils, a correction factor of 1.3 is required to calculate organic carbon (C) levels. Bremner and Jenkinson (1960), however, have reported that this factor is not required for C assays on plant material. Because the method was required to measure increasing algal mass, and because soil C contribution remained constant over the experimental period, the correction factor was not used.

Using the procedure as published (Bremner and Jenkinson, 1960) on oven-dried algal samples, a direct relationship could not be obtained between the measured organic C and algal dry weight (Table 3.1).

Table 3.1 Effect of increasing algal mass on the amount of oxidizable algal carbon

Algae (mg dry weight)	Carbon/dry weight
0.9	0.716
1.8	0.675
3.6	0.583
4.0	0.368

Incomplete oxidation of the larger algal samples was responsible for the decreasing ratios. In order to facilitate complete oxidation of the

samples, the effect of more rigo rous oxidizing conditions was evaluated. Four duplicate algal samples and soil samples were heated with acidified potassium dichromate for varying time periods. The result of heating the oxidizing mixture at 100°C for periods up to 3hr are shown in Fig. 3.1. The data indicate that only 50% of the algal C was oxidized without heating; this produced a C/dry wt ratio of 0.368. The maximum reading for algal C was attained only after 2hr of heating at 100°C, producing a C/dry wt ratio of 0.743. The measured level of soil organic C continued to increase, even up to 3hr of heating. At this point the measured C value of the soil was larger than unheated value by a factor of 1.3. This indicates that oxidation was essentially complete. The attainment of stability of the soil C value was unimportant, because it did not vary significantly during an experiment. Heating did not affect the blank reading of the oxidizing mixture. Consequently, by heating the oxidizing mixture at 100°C for 3 hr it was possible to produce a reliable value for algal C content. The heating procedure was incorporated into all subsequent C determinations. The procedure adopted was as follows: duplicate 10-ml aliquots of the algal culture were dried overnight at 100°C in 250-ml Erlynmeyer flasks. A 10-ml aliquot of 1N potassium dichromate and 20ml of concentrated H_2SO_4 were added and the mixture was heated at 100°C for 3hr. The cooled digestion mixture was diluted to 200ml with distilled water, 10 ml of syrupy phosphoric acid was added, and the residual potassium dichromate determined by titration with 0.1N ferrous ammonium sulphate. A 2% solution of barium diphenylamine sulphonate was used as the end-point indicator.

3.1.6.2 Chlorophyll concentration, organic carbon, and dry weight

The measurement of chlorophyll a concentration (hereafter

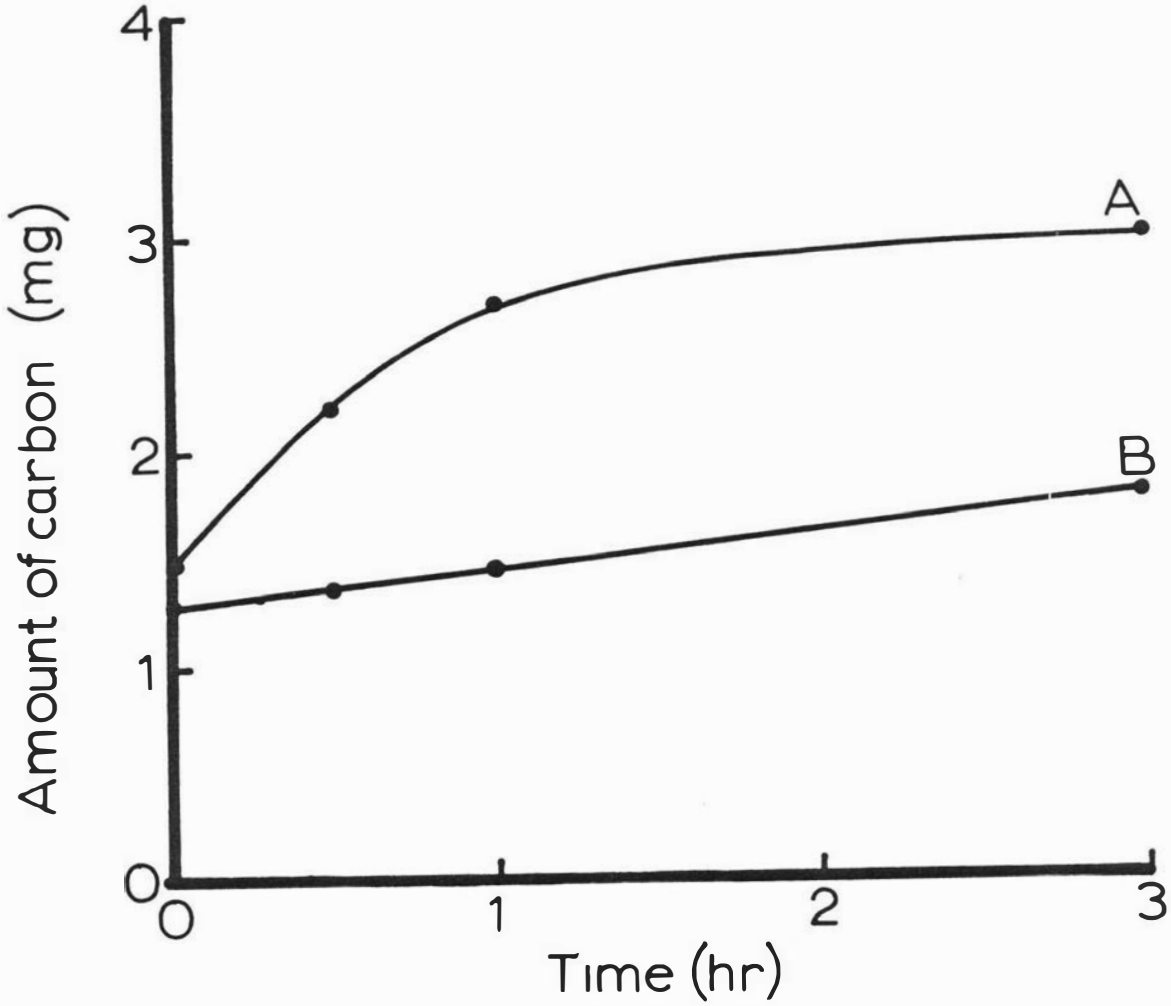


Fig. 3.1 Effect of time of heating the oxidising mixture at 100°C on the determined carbon content of Anabaena (4 mg, A) and of < 30-µm soil material (40 mg, B).

referred to as chlorophyll, or chl when abbreviated) has been used in several studies to estimate the quantity of phytoplankton present (see Section 2). As an estimate of algal mass, it has advantages in that it is quick and simple to perform and sensitive to small amounts of algae. Based on a review of the literature, the method of Talling and Driver (1963) was adopted. This allows for the calculation of chl in an ethanol extract, from absorbance in the region of 663-665nm, by the approximate relationship chl ($\mu g\ ml^{-1}$ solvent) = 13.9 x D_{665} (pathlength cell 1cm, D = absorbance at 665). This method was evaluated using Anabaena in the presence of soil material. Aliquots of Anabaena cells were collected on a membrane filter (0.45 μm pore size) and extracted with 20ml of ice-cold ethanol containing 0.1% $MgCO_3$ for 24hr in the presence of 0.5g of 30-μm soil material. The results (Table 3.2) show that 0.5g of soil material had no significant effect on the chl extracted. Because much smaller weights of soil would be present in aliquots taken from culture flasks,

Table 3.2 Effect of increasing algal mass and soil on extractable chlorophyll concentration

	Aliquot of algae (ml)	Absorbance (663nm)	Chlorophyll ($\mu g\ ml^{-1}$ solvent)
	10	0.078	1.08
	15	0.116	1.61
	20	0.153	2.13
	30	0.218	3.03
0.5g soil	10	0.075	1.04
"	30	0.217	3.02
"	0	0.002	0.02

the chlorophyll extraction procedure was seen to fulfil the requirements of an algal biomass indicator for the soil-algal systems. It was necessary, however, to further evaluate the applicability of chl as an indicator of biomass, because cell pigment content would depend on the physiological condition of the cell, and also to determine what further information could be obtained by the continuous monitoring of chl over a period of algal growth. Firstly, assuming algal C content to be an absolute measurement of biomass, unaffected by cell nutrition, a comparison was made between chl and C content during the exponential growth phase of the alga Anabaena subcylindrica, grown in a surplus of P. Carbon levels were determined on 20-ml aliquots of the algal culture to enhance the accuracy of the method. A 5-ml aliquot was used for chl determination. The results are shown in Fig. 3.2. A linear relationship was obtained between the two parameters over the period of exponential growth (i.e., up to the point before nutrient deficiency (Healey, 1973)). Therefore, it was concluded that chl gave a reasonable measure of algal production under conditions where growth was not nutrient limited.

Algal chl was further investigated under varying degrees of P limitation in order to evaluate its use with algal cultures where soil P was the only source of P. Five P treatments (0, 0.24, 0.36, 0.60, and 0.84 mg P l^{-1}) were duplicated in ten 2-litre culture flasks, containing 1 litre of ASM-1, N- media. These were adjusted to pH 7 and incubated overnight at 28^{o}C, before being inoculated with minimally P-starved algae. The flasks were incubated under liquid culture conditions until algal growth ceased. Over the growth period, chl, algal dry weight, and alkaline phosphatase activity were monitored regularly. The results are shown in Fig. 3.3a, b, and c. Both chl (Fig. 3.3a) and algal dry weight (Fig. 3.3b) were clearly limited by P concentration; maximum yields were

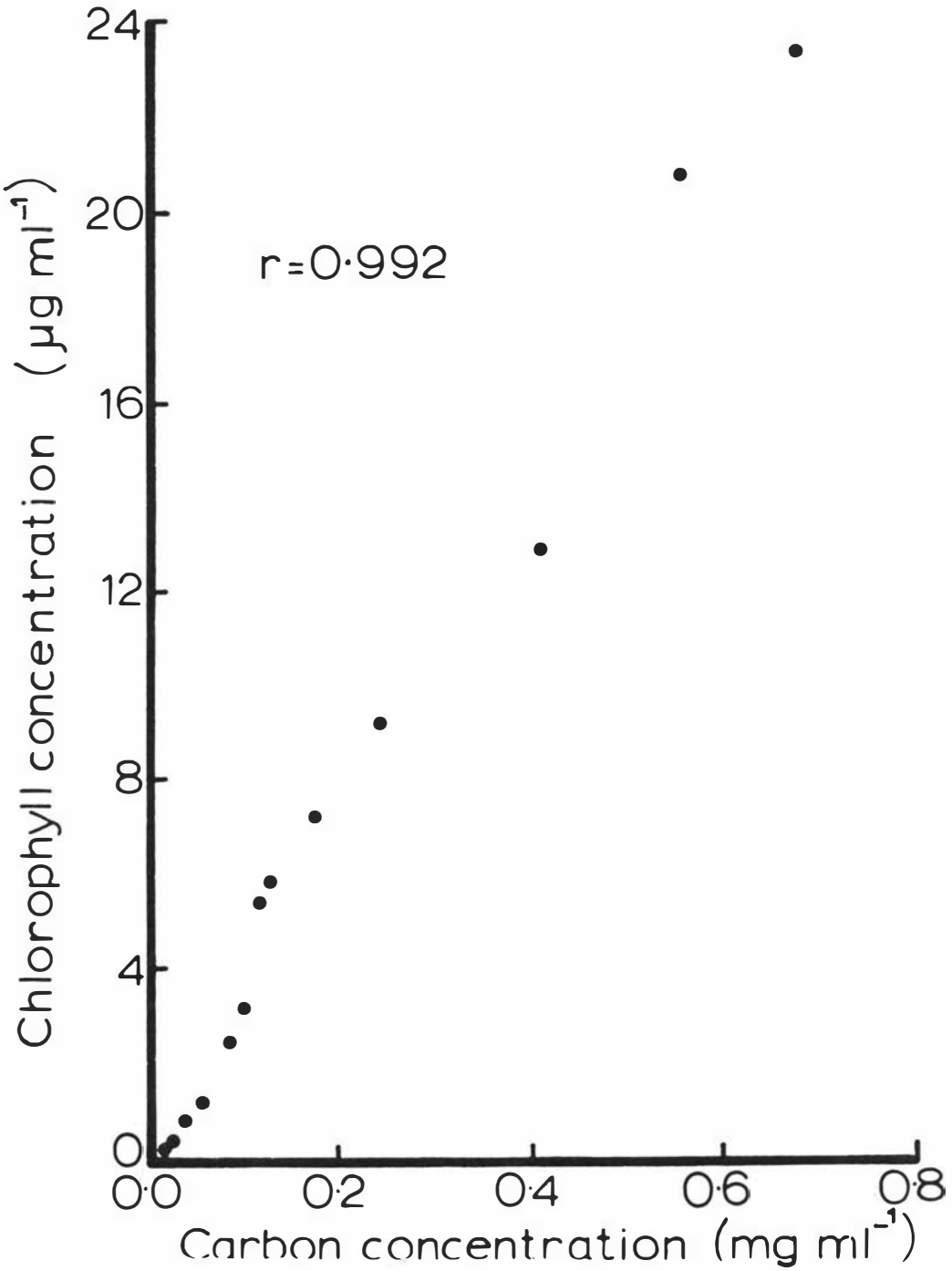


Fig. 3.2 Relationship between chlorophyll and carbon concentrations during the exponential growth of Anabaena.

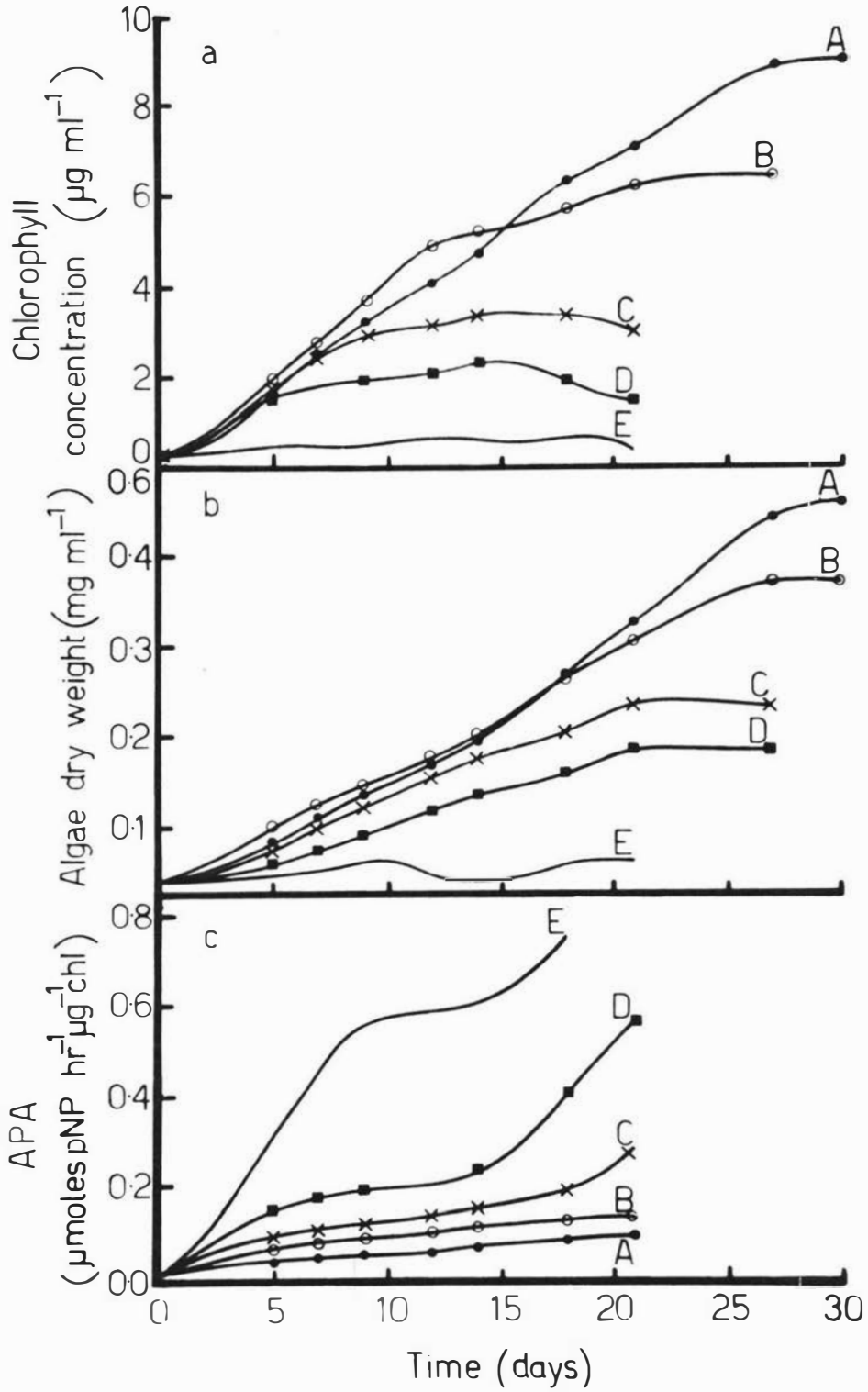


Fig. 3.3 Chlorophyll concentration (a), dry weight of algae (b), and alkaline phosphatase activity (APA) (c), during the growth of Anabaena in ASM-1, N-media at varying P concentrations (A = 0.84, B = 0.60, C = 0.36, D = 0.24 and E = 0 mg P l^{-1}).

more affected than the rate of dry matter or chl production, especially at the higher P concentrations of 0.60 and 0.84mg P l^{-1}. The dramatic inducement of whole-cell alkaline phosphatase activity by P limitation (Fig. 3.3c) is consistent with the findings of Healey (1973). This induction was clearly seen in the 0.24mg P l^{-1} system after 15 days and in the 0.36mg P l^{-1} after 17 days. The algae in the 0.60 and 0.84mg P l^{-1} systems did not apear to become as severely P starved during the experimental period studied. It was hoped that in future studies, alkaline phosphatase activity could be used to follow the P status of the test algae during growth on particulate materials.

In all P treatments, chl reached maximum levels (Fig. 3.3a) and began to decrease before dry matter production decreased (Fig. 3.3b). Healey (1973) found the same response with P-limited Anabaena. Consequently chl was an earlier indicator of P limitation than dry weight. When plotting chl against algal dry weight for each treatment it was found that no simple relationship existed between the two parameters over the period of algal growth (Fig. 3.4). There did, however, appear to be three distinct stages, for each P treatment, during which separate linear relationships existed between chl and dry weight. Consequently, no general relationship could be found to relate chl to dry weight, except to say that chl appeared to be governed by two factors, algal mass and P availability. When P availability was constant, a linear relationship probably existed between chl and algal dry weight.

For chl or dry weight to be used as a biomass indicator for P limited studies, it was necessary to determine whether maximum chl or maximum dry weight in each flask was related to the total amount of available P present. The chl or maximum dry matter produced was plotted against the available P concentration in each system. Regression lines

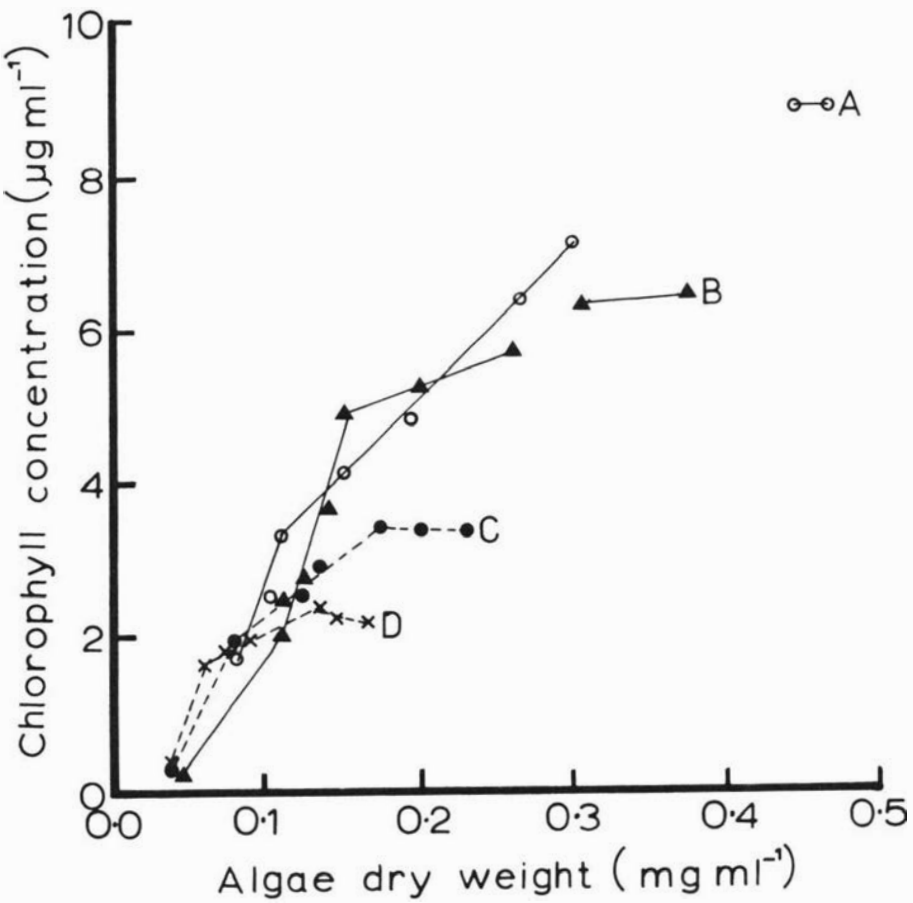


Fig. 3.4 Variation in the chlorophyll concentration-algae dry weight relationship during the growth of Anabaena in ASM-1, N- media at varying P concentrations (A = 0.84, B = 0.60, C = 0.36, and D = 0.24 mg P l^{-1}).

and the variance and correlation coefficients were calculated for each set of data (Fig. 3.5). There was a high correlation between maximum chl and total available P (r = 0.998), and maximum dry weight and total available P (r = 0.995). It was evident that the chl data were subject to a slightly lower deviation from a direct relationship, having a variance of $\sigma^2 = 0.30 \mu g^2 ml^{-2}$ compared to the dry weight data variance of $\sigma^2 = 0.33 mg^2 ml^{-2}$.

3.1.6.3 Adopted method of biomass measurement

Although the reliability of the C determination was improved by heating the digestion mixture, the lengthened procedure was too time consuming to fit into a 48-hr sampling interval which involved a P fractionation procedure and an alkaline phosphatase activity determination. Also, an experimental culture volume of 1 litre dictated the necessity for small sample aliquots in order to allow a number of sampling events. Small aliquots (< 10ml) would reduce the amount of measurable C and therefore the accuracy of the titrations. For large concentrations of algae the method was still reliable, but 1-g samples of soil would not produce sufficiently large amounts of algal material. For the same reasons, algal dry weight was not a reliable indicator of biomass. In the presence of soil, sub-sampling errors produced variable weight recordings because the soil consituted by far the major part of the recorded weight. On the other hand, chl was easily measured and was more sensitive to changes in algal mass. For example, there was a 37-fold chl increase in the 0.84mg P l^{-1} system (Fig. 3.3a) but only an 8-fold increase in dry weight over the same growth period. The chl was easily determined in the presence of soil and the data were highly correlated with the amount of available P present; this would allow growth on soil P to be compared with growth on known amounts of P. Also, a sample size

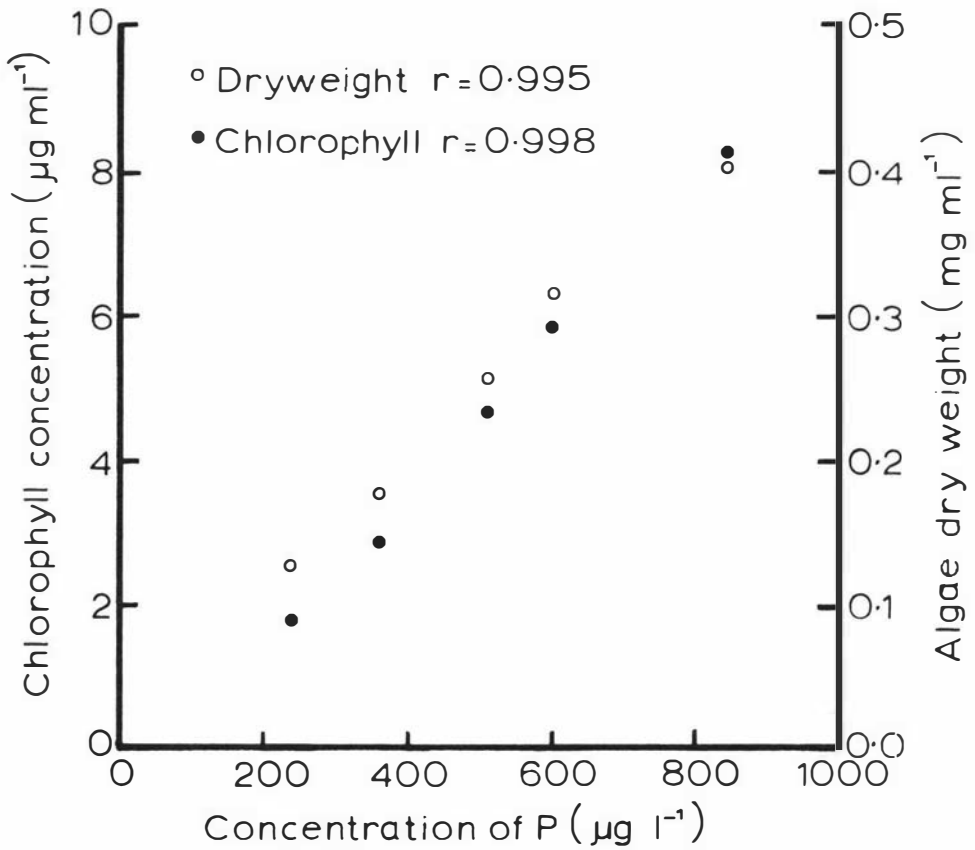


Fig. 3.5 Relationship between maximum chlorophyll concentration or maximum dry weight of Anabaena and the available P concentration of the growth media.

of only 5ml was required to produce reliable results and therefore several sampling events could be undertaken over the period of algal growth without greatly reducing the culture volume. Chlorophyll extraction, measurement, and expression as μg chlorophyll ml^{-1} culture (μg chl ml^{-1}) was adopted as a reliable indicator of algal mass up to the point of pigment deterioration.

3.2 Phosphorus Determination

Inorganic P (IP) removed by a range of extractants was measured in neutralized aliquots of each extractant by the method of Murphy and Riley (1962).

Total extractable P (TP) was measured using the same method after persulphate digestion (Environmental Protection Agency, 1971) of a suitable aliquot of each extractant. Extractable organic P (OP) was calculated as the difference between extractable TP and IP. The absorbance of the phosphomolybdate complex (Murphy and Riley, 1962) was measured at 712 nm using a Pye Unicam SP 1800B spectrophotometer.

3.2.1 Definition of terms

The term P generally refers to the orthophosphate ion, but may be qualified by the terms organic (OP) and inorganic (IP). Where the term available-P or extractable-P is used, P refers to both organic and inorganic P.

The term TP refers only to that P measured in the persulphate digestion of a soil extract (see Section 7).

The terms 0.1M NaOH-IP, 0.1M NaOH-OP etc. refer to the amount of IP or OP etc., extracted by the named reagent, in this case 0.1M NaOH. This term may be further qualified by the words algal or soil, thereby signifying the source of the extracted IP, OP etc. (e.g. 0.1M NaOH-algal-P).

3.2.2 Phosphorus fractionation procedure

3.2.2.1 Solution-P

A 40-ml aliquot was pipetted from the culture flask (soil: solution ratio 1:1000) into a 40-ml polypropylene, screw-top centrifuge tube and centrifuged at 27000 x G for 10min at -4°C (Sorvall Superspeed RC-2B). It was found that the algal cells formed a more compact residue at this low temperature. The supernatant solution was decanted through a membrane filter (Millipore 0.45μm) and IP and TP were determined on suitable aliquots.

3.2.2.2 Sodium hydroxide-extractable-P

A 30-ml aliquot of a solution containing 0.1M or 1M (NaOH + NaCl) was added to the residue and the tube was shaken on an end-over-end shaker for 17hr at 24°C, centrifuged at 27000 x G for 10min at 0°C and decanted through a membrane filter (Millipore 0.45μm). The residue was washed with 20ml of 1M NaCl, centrifuged, and the washings combined with the NaOH extracts. The extracts were coloured to different degrees, depending on the amount of humic material present in the soil. This colour was found to interfere with the development of the phosphomolybdate blue colour and in some cases humic precipitates formed on addition of the acidic Murphy and Riley reagent. In general, the cloudy solutions gave higher and often non-reproducible absorbance readings. After short experimentation, it was found that adjustment of the NaOH extract to pH 1 allowed precipitation of the humic acids, which removed the interference with phosphomolybdate development, and afforded the greatest recovery of IP. At higher pH values (pH 3 and 7), IP was also precipitated with the humic acids. The procedure adopted for measuring NaOH-IP was as follows: The pH of the filtered NaOH extract (10ml) was adjusted to pH 1 with a

calculated volume of 1M HCl and immediately centrifuged to avoid possible acid hydrolysis of organic P; this precipitated a large quantity of the humic acids, which allowed a clear phosphomolybdate blue colour to develop with maximum recovery of IP. NaOH-TP was determined by persulphate digestion of the filtered extract.

3.2.2.3 Citrate-dithionite-bicarbonate extractable-P (CDB-P)

A 10-ml aliquot of 0.27M sodium citrate/0.11M sodium hydrogen carbonate was added to the residue, which was heated to 80°C in an oven. Sodium dithionite (0.3g, fresh, solid) was added and the tube shaken for 30sec and replaced in the oven for 20min; the tube was shaken at 5-min intervals. The tube was cooled to room temperature, centrifuged at 27000 x G for 10min, and the supernatant solution was decanted into a 100-ml polyproplene, press-cap centrifuge tube. The residue was washed with 1M NaCl (20ml), centrifuged, and the washings combined with the CDB extract and the volume adjusted to 30 ml. An air-bubbling system was placed in each 100-ml tube and moist air was bubbled through the combined washings and extract for 4hr, or until an oxidized state was achieved, as indicated by the colour change from colourless to yellow. The method of Weaver (1974) was used to overcome citrate interference when determining the IP concentration of the extract except that the Murphy and Riley reagent (Murphy and Riley, 1962) was used instead of reagent B of Watanabe and Olsen (1965), for the colorimetric determination of IP concentration.

3.2.2.4 Acid-extractable-P (1M HCl-P)

A 10-ml aliquot of 1M HCl was added to the residue and the tube shaken for 4hr on an end-over-end shaker at 24°C. The supernatant solution was decanted after centrifugation, and the IP concentration was measured in a neutralized aliquot.

SECTION 4

EFFECT OF PHOSPHORUS AVAILABILITY ON EXTRACTABLE ALGAL CELL PHOSPHORUS

4.1 Introduction

In bioassay studies which attempt to determine the amount and forms of biologically-available P in particulate material, the problem of not being able to re-separate the test algae from the particulate P source is encountered. It is therefore impossible to directly determine those soil P fractions that have been depleted by algal growth, because algal P is simultaneously extracted with the soil P fractions during chemical fractionation procedures. Golterman et al. (1969) attempted to overcome this problem by assuming that the cell P fraction in Scenedesmus obliquus remained constant during growth. Consequently, he equated the amount of P extracted from a known biomass which was produced in a solution of known P concentration, with the biomass produced by the P derived from lake mud and then subtracted the estimated extractable-algal P from the mud plus algae extraction value. This permitted the determination of the amount of P remaining in a specific soil P fraction. Sagher (1974) found that the cell P content of Selenastrum capricornutum and Nostoc sp. decreased during growth for all media P concentrations. In order to correct for algal P in sediment-algal systems, a correction factor which was based on the extraction of P from algae during the stationary phase of growth was used. During the early stages of growth, this would lead to an underestimation of the correction for algal P. Because algal P content is known to vary greatly according to P availability (Healey, 1973; Keenan and Auer, 1974), it is difficult to produce accurate algal correction figures for the whole algal growth period, during which P

availability is believed to decrease greatly. In order to produce accurate correction data for algal P, especially for P-assimilating algae, the control systems must have a similar P environment to the particulate system, i.e., a low concentration of solution P which is buffered to a large extent by P sorbed at the soil surface. Algal growth-control flasks containing P solutions do not adequately fulfil these requirements. Because the P would be directly available, the algae could rapidly and completely assimilate solution P. Further growth would be dependent on stored P, and not on P being slowly released to solution and thus available to the algae.

It is well known that hydrous ferric oxide gel(Fe gel) has P sorption characteristics which are qualitatively similar to those of soils (McLaughlin et al., 1977; Ryden et al., 1977a). Theoretically, therefore, P sorbed by Fe gel to give a certain saturation of the sorption maximum could produce a similar P environment to that of a soil-solution system. These systems could fulfil the requirements of the algal growth-control flasks in studies of the availability of particulate P. Algae grown in a similar P environment to a particular soil could then be fractionated chemically to provide more accurate estimates of the algal P contribution to the P fractions of a soil-algal system.

This section reports on the variability of the cell P content of Anabaena with changes in P availability and evaluates the potential of using Fe gel to control P concentrations in algal growth studies. A modification of the soil inorganic P fractionation procedure developed by Chang and Jackson(1957) and modified by Williams et al.(1967) was to be used to characterise and monitor the depletion of soil P. Golterman et al.(1969) had previously indicated that negligible P was removed from algal material by the reagents used subsequent to the NaOH extraction. Consequently,

0.1M NaOH was used to study the extraction of algal P, because it was considered that this fraction would probably contain the largest algal P contribution to the P extracted from soil + algal systems in later studies.

4.2 Materials and Methods

4.2.1 Preparation of Fe gel

A solution of 0.4M $Fe(NO_3)_3$ was adjusted to pH 7 with 1M NaOH during constant stirring. Subsequent additions of 0.1M NaOH were made over a period of 1 hour to maintain a pH of 7. The neutralized solution was left overnight and the Fe gel recovered by centrifugation (2000 r.p.m. IEC UV Centrifuge). The precipitated gel was washed with distilled water until the gel ceased to flocculate during centrifugation at 1000 r.p.m. for 15 min. The volume of gel was adjusted to give a suitable working concentration (10 mg ml^{-1}). Aliquots (10 ml) of the Fe gel were shaken in 100-ml polyproplene tubes with 5 ml of stock (x 10 concentration) ASM-1, P-, N- media and different amounts of K_2HPO_4 in a total volume of 50 ml. The pH was adjusted to pH 7 until it was stable. After 4 days the gel was separated from the supernatant solution by centrifugation. The P concentration of the supernatant was determined and the amount of P sorbed by the Fe gel was calculated. Several samples of Fe gel to which different amounts of P had been added to give solution P concentrations similar to those maintained by soils, were resuspended in 10ml of ASM-1, P-, N- media and washed into knotted dialysis tubing with a further 5ml of growth media. The dialysis tubing plus Fe gel was then used as a P source in algal-culture flasks. The flasks were shaken for 2 days prior to the inoculation of algae, to allow equilibration of the system and measurement of the level of P sustained in solution by the phosphated gel.

4.2.2 Soil materials

The < 30-μm particle size fractions of Waiotu brown loam (0-5cm) and Manawatu silt loam (0-5cm) were separated and recovered as described in Section 6. The soils are also described more fully in Section 6. Samples (1g) of these materials were suspended in ASM-1, N- media as the sole P source. The Manawatu river sediment was collected from the river during a flood and was concentrated by continuous centrifugation from a large volume; no particle-size separates were removed and 1g of the whole sample was resuspended in ASM-1, N- media.

4.2.3 Experimental procedure

Phosphated Fe gels were prepared to provide a range of solution P concentrations encompassing those sustained by the soil material (soil: solution ratio = 1:1000). The highly-fertilized Manawatu silt loam, however, was not catered for, being an extreme example (Table 4.1). In the systems without Fe gel, K_2HPO_4 was added to give solution P levels which were expected to be similar to the amount of biologically-available soil P (0, 220, 660, and 880 μg P l^{-1}). All flasks were adjusted to pH 7, shaken for 2 days, and the solution P concentrations determined (Table 4.1). The flasks were then incubated at 28° overnight before being inoculated with minimally P-starved Anabaena. Liquid culture conditions were maintained until algal growth ceased. Over the growth period, whole cell alkaline phosphatase, chlorophyll concentration, and 0.1M NaOH-extractable TP levels were monitored. To confirm that algal growth had ceased due to P starvation, 500 μg P (K_2HPO_4) was added to re-establish growth. This was achieved in most flasks where P starvation had not been sufficiently severe to cause death. Although it could not be confirmed, it was assumed that P starvation had caused

Table 4.1 Solution IP concentrations during the growth of Anabaena in Fe gel and soil systems and in P solutions

System	P added (μg)	Solution IP (μg l^{-1}) at time (days)			
		0	1	5	8
		Fe gel			
A	0	< 1	< 1	< 1	< 1
B	2496	2	< 1	< 1	< 1
C	2990	5	< 1	< 1	< 1
D	3460	36	7	< 1	< 1
		Soil			
River sediment	-	7	< 1	< 1	< 1
Waiotu	-	2	< 1	< 1	< 1
Manawatu	-	126	61	< 1	< 1
		Solution			
E	0	< 1	< 1	< 1	< 1
F	220	221	40	< 1	< 1
G	660	660	213	4	< 1
H	880	850	361	4	< 1

the cessation of growth in all flasks because all other nutrients were adequately supplied.

4.3 Results and Discussion

4.3.1 Solution P concentrations

Although Fe gel maintained low solution P concentrations, similar to those maintained by the River sediment and Waiotu soils, large amounts of P remained sorbed by the Fe gels (Table 4.1) indicating the strong P-buffering capacity of the system. These low dissolved P concentrations were rapidly depleted by the P-starved Anabaena. As discussed later, however, the continued increase in extractable algal-TP (Fig. 4.1a) confirmed the fact that the Fe gel was acting as a reservoir, slowly releasing P to solution. In all flasks, solution P was rapidly assimilated by the algae. This has been referred to as luxury uptake by Keenan and Auer, (1974), and as over compensation of P deficiency by Kylin (1964) and Healey (1973). Regardless of whether this was a P storage response or a compensation response, P was concentrated in the cells to levels far greater than the extractable P content of exponentially-growing cells (Fig. 4.1b, 4.2b). In flasks G and H (Table 4.1), which had higher initial P concentrations, a second slower P uptake rate followed the initially rapid rate. In this case, it is probable that the P uptake rate was being limited by large P stores within the algae (Azad and Borchardt, 1970).

4.3.2 Biomass as measured by chlorophyll concentration

Overall, the algal chl concentration data showed the trends of growth dictated by different levels of available P. All solution P cultures

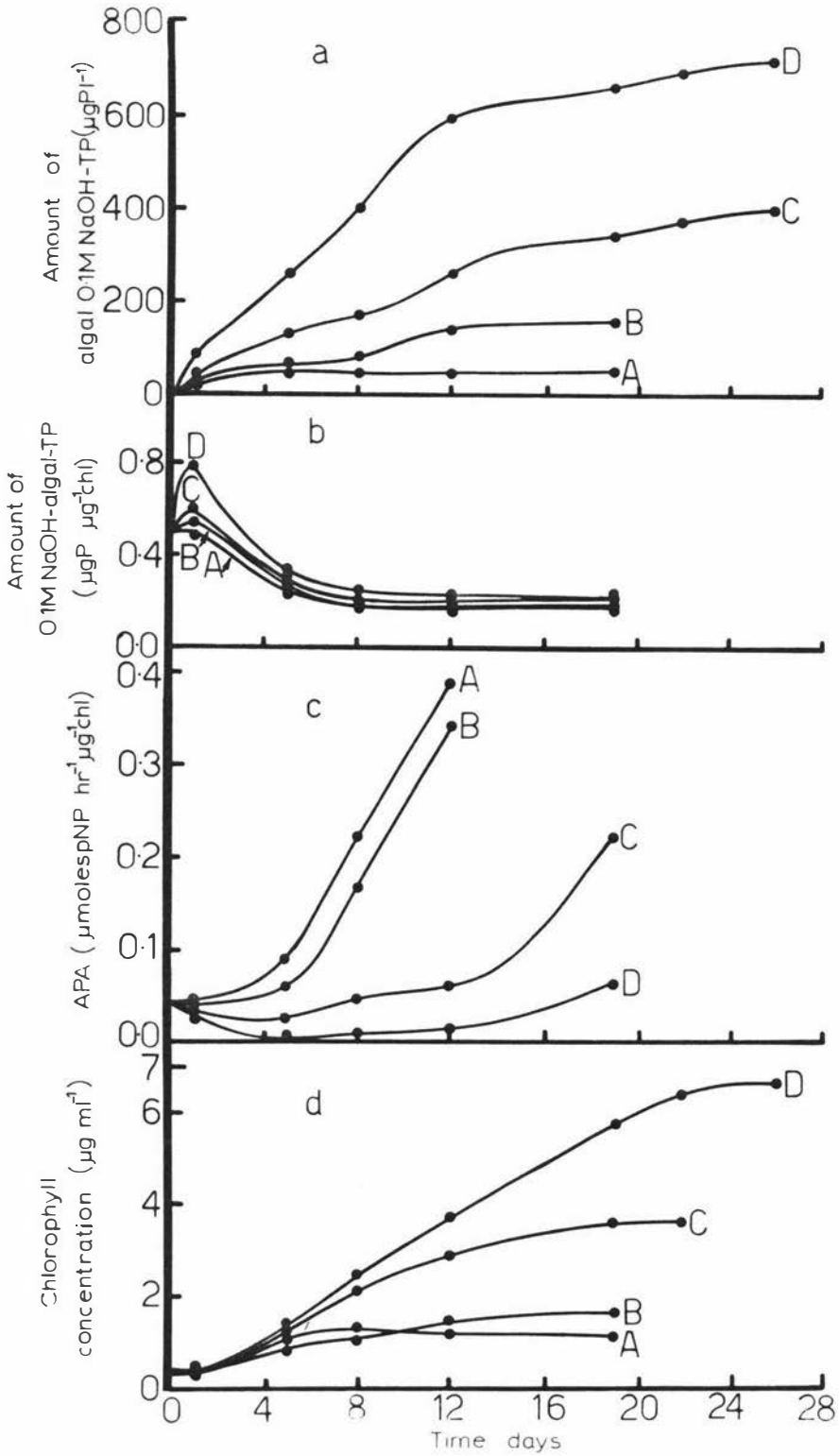


Fig. 4.1 Amount of algal 0.1M NaOH-TP over and above that in the inoculum (a), amount of 0.1M NaOH-algal-TP per unit biomass (b), alkaline phosphatase activity (APA) (c), and chlorophyll concentration (d) during the growth of Anabaena in Fe gel systems of increasing P content (A = 0, B = 24.9, C = 29.9, and D = 34.6 µg P mg^{-1} Fe gel).

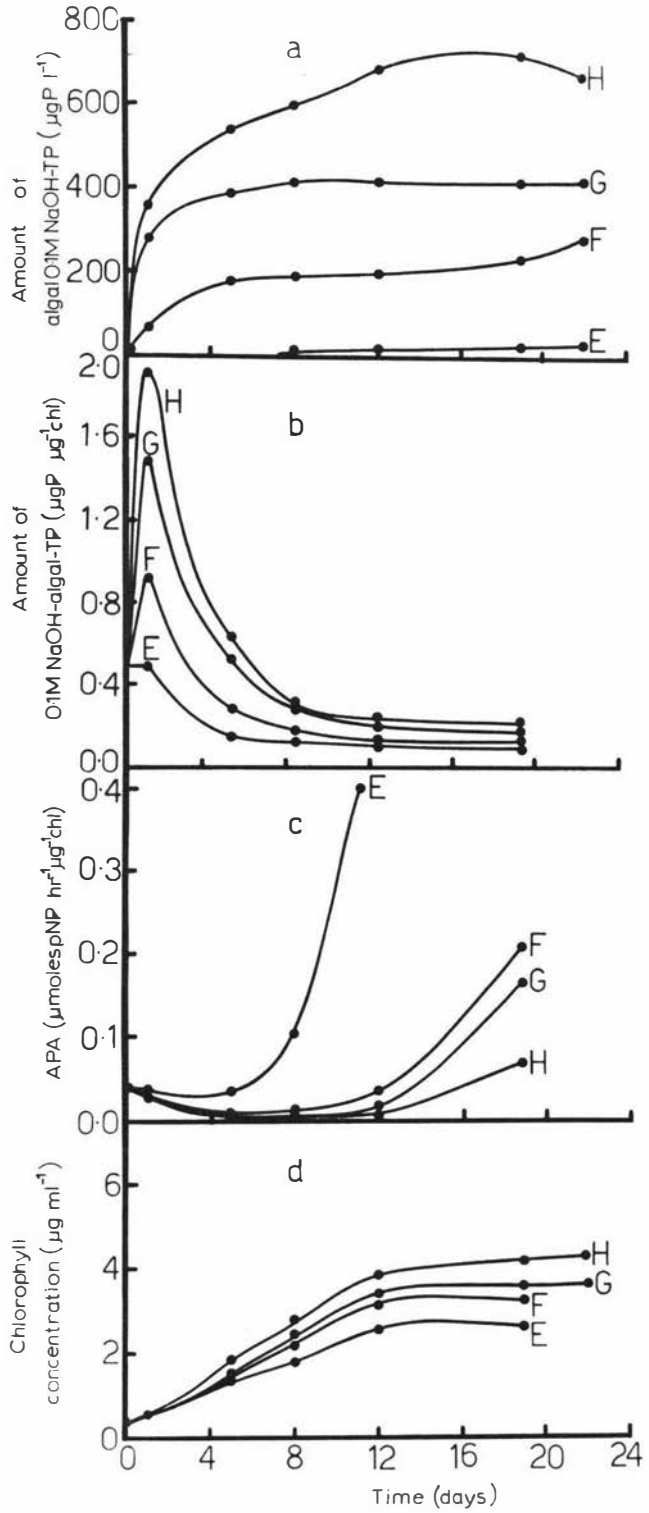


Fig. 4.2 Amount of algal 0.1M NaOH-TP over and above that in the inoculum (a), the amount of 0.1M NaOH-algal-TP per unit biomass (b), alkaline phosphatase activity (APA) (c), and chlorophyll concentration (d), during the growth of Anabaena in solutions of increasing P content (E = 0, F = 220, G = 660, and H = 880 µg P l^{-1}).

(F, G, and H, Fig. 4.2d), had similar initial growth rates up to 5 days. Because most of the solution P in each flask had been assimilated by day 5, it could be assumed that algal growth was no longer dependent upon solution P but on P stored in the algae. Thus, initial growth rate may have been purely a function of the rate of metabolism of stored P. It was noticeable that growth rates decreased (Fig. 4.2d) as the extractable cell P content decreased (Fig. 4.2b). The final amount of growth (chl production) was linearly related to the total amount of P added to each flask. Growth in the P-flask E was the result of the algal inoculum not being sufficiently P starved. This became apparent once it was found that extractable-algal-TP in flask E decreased from the time of inoculation (Fig. 4.2b). The phosphated Fe gel systems did not produce similar initial growth rates. Growth rates and chl production were positively related to the amount of P present in the system. Only with the most highly phosphated Fe gel (D) did the exponential growth rate reach that of the solution P systems F, G, and H. Thus it could be assumed that in Fe gel systems B and C, growth was P limited from as early as day 1. This point was reinforced by the algal-TP content data (Fig. 4.1b) which decreased to below that of the inoculum by day 2. Growth in the Fe gel, P- flask (A) was considerably less than in the solution P- flask (E) and could have resulted from increased mechanical shaking because of the presence of the dialysis tubing, or from the Fe gel sorbing nutrients from the ASM-1 media. These effects did not prevent the algae from reaching maximum growth rates with highly phosphated gel (D), however, and thus they did not affect the principle of the experiment. A particulate P source material, such as fine soil particles would also sorb ions from a nutrient media, and therefore the

Fe gel may compensate for this to some extent in the control growth flask.

Anabaena grew rapidly on the two soils and suspended river sediment; total growth in all three cases was far greater than growth which could have been sustained by solution P levels alone (Fig. 4.3a). The growth rate of the Anabaena on the highly-fertilized Manawatu silt loam exceeded the growth rates in the control flasks and this suggested that the presence of other nutrients may have been important. Growth curves, for the river sediment and the Waiotu soil (Fig. 4.3a) followed similar patterns to growth in the Fe gel flasks C and D (Fig. 4.1c).

4.3.3 Algal P status

The P status of Anabaena during growth was evident from the 0.1M NaOH-algal-TP content and alkaline phosphatase data (Fig. 4.1b and 4.1c; 4.2b and 4.2c). Although alkaline phosphatase activity is not a universally applicable measure of algal cell P status (Keenan and Auer, 1974), it has been shown that the enzyme does reflect to some extent the cell P status for species of Anabaena (Healey, 1973). No simple correlation exists, however, between enzyme activity and algal P. In Fig. 4.2a and 4.2b, luxury P uptake is clearly demonstrated. Extractable algal-TP rapidly reached a maximum level which remained fairly stable over the whole growth period (Fig. 4.2a). In the case of flasks E and F, the increase in extractable P at the end of the growth period was associated with a loss of pigmentation and a general deterioration of the algal cells. The extractable algal-TP data for the P solutions F, G, and H, show that after the initial rapid assimilation of P (Table 4.1), subsequent algal growth must be dependent on internal stores of P. The extractable TP per unit of biomass (Fig. 4.2b) decreased rapidly over the

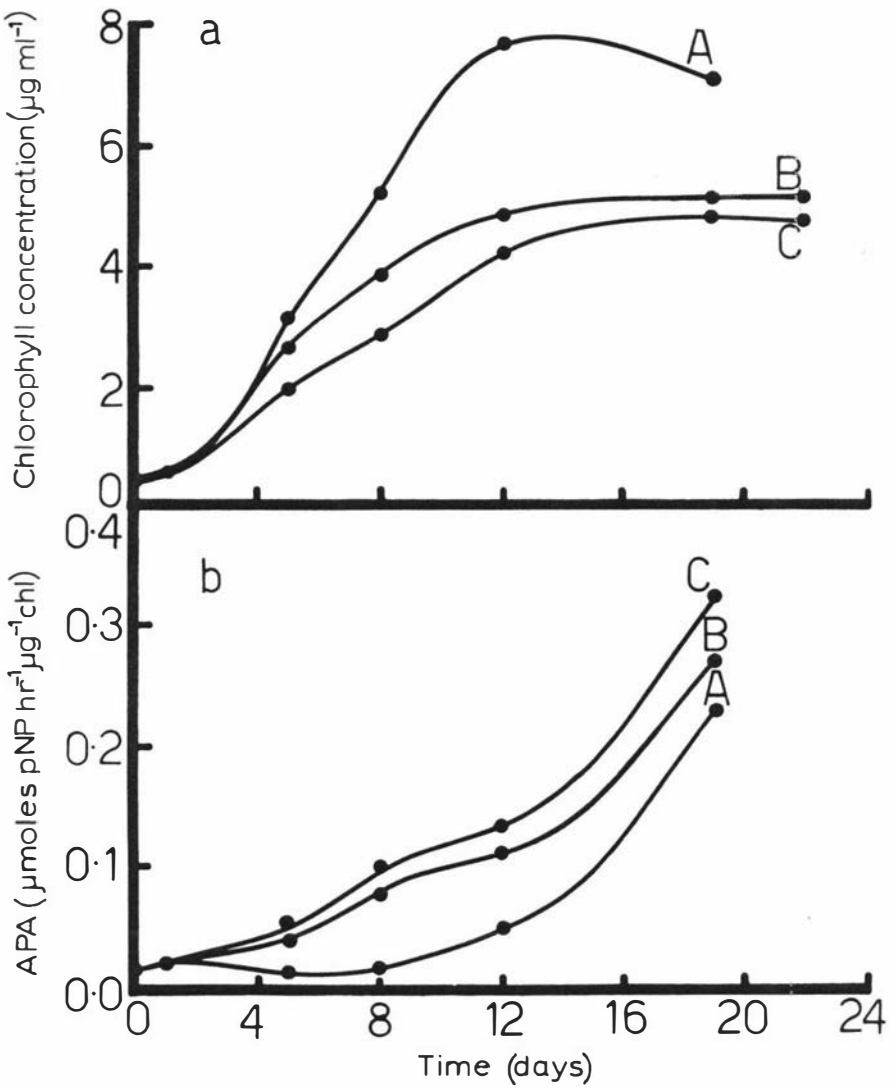


Fig. 4.3 Chlorophyll concentration (a) and alkaline phosphatase activity (APA) during the growth of Anabaena on the < 30-µm material from Manawatu (A) and Waiotu (B) soils, and on Manawatu River sediment (C).

exponential growth period, the stationary phase of growth being reached (Fig. 4.2c) when extractable algal-TP content reached a minimum level between 0.091 and 0.22 µg Pµg chl^{-1}. At this point, the whole cell alkaline phosphatase activity increased markedly. Several workers have already established that the induction of the enzyme is related to P limitation (Price, 1962; Kuenzler and Perras, 1965; Healey, 1973).

Extractable algal-TP (Fig. 4.1a) increased at a much slower rate in the Fe gel systems, than in the solution P systems (Fig. 4.2a). The initial rates for each individual P treatment also varied, although these trends were less obvious with the solution P systems. The difference in extractable-TP levels per unit of biomass for the separate Fe gel treatments A, B, C, and D (Fig. 4.1b), was small compared to that for the solution P treatments E, F, G, and H (Fig. 4.2b), indicating some control of algal P uptake. Having established that Anabaena rapidly assimilates dissolved P, as shown by the data obtained for the solution P systems, it was clear that P supply to the inoculated and growing algae was limited by the rate of desorption and diffusion of P from the gel system. The slower rates of accumulation of extractable algal-TP (Fig. 4.1a) presumably described the rate of release of P from the Fe gel systems which, in turn, dictated the growth patterns shown in Fig. 4.1d. Although there was considerable algal growth in the Fe gel systems, growth was limited from the time of inoculation, by the supply of P. The alkaline phosphatase data (Fig. 4.1c) clearly demonstrated this feature because in all flasks, the enzyme activity began to increase after the fifth day, at which point extractable algal-TP per unit of biomass was well below that of the inoculated algae (Fig. 4.1b). This reinforces the point that most of the algal growth in the Fe gel systems occurred under conditions of P limitation.

The alkaline phosphatase data recorded for the soil cultures (Fig. 4.3b) followed more closely the patterns exhibited by the algae grown in the Fe gel systems, than in the P solutions. Enzyme activity in all soils began to increase after 5 days. Because it has been shown that enzyme activity was closely related to the level of extractable algal-TP per unit of biomass, it was assumed that the P content of algae grown in a soil system was more similar to algae grown in an Fe gel system. It was also assumed that the affinity of the soils for P would produce conditions where the availability of P for algal growth was restricted to some extent, as in the gel systems.

4.4 General Discussion

The results obtained clearly demonstrate the P-assimilating ability of the test algae Anabaena, and also that P availability governs the amounts of algal cell P. This is in agreement with the findings of Healey (1973) and Keenan and Auer (1974). As also indicated by the results of Healey (1973), alkaline phosphatase activity appeared to describe the algal cell P status quite effectively; increases in activity were observed when extractable algal cell P dropped below approximately 0.27 $\mu g\ P\ \mu g\ chl^{-1}$. In order to obtain correction data for the fractionation of soil-algal mixtures, it is evident that the algae in the soil mixture and in the control flask must have similar P contents, otherwise incorrect estimates of the algal contribution to soil-algal extractable P would be made. It would appear from the data obtained that algae which have similar chlorophyll (i.e. biomass) and alkaline phosphatase levels, and are grown in similar P environments will have similar cell P contents. The initial solution P concentrations of the

Fe gel systems were comparable to the dissolved P concentrations maintained by the soils. Consequently, the amounts of P initially taken up by the algae in the two systems must be similar when the same weight of algae is present. On the other hand, algae grown in a P solution initially had large P stores, because of luxury uptake (Fig. 4.2b). Thus, whereas the algae grown in a P solution had similar biomass (chlorophyll concentration) to the algae grown on the soil during the first 5 days (Fig. 4.2d, 4.3d), their internal P contents would be vastly different. If samples of the algae grown in a P solution were used to obtain a correction for algal P contribution to the 0.1M NaOH-P fraction of soil-algal systems during the initial stages of growth, a gross overestimate would be made. The average P content of algal cells during the stationary phase of growth was far less variable than during the initial growth phase (Fig. 4.1b, 4.2b). The variability was still sufficient (0.15 to 0.22 μg $P\mu g^{-1}$chl) for Fe gels and 0.091 to 0.22 μg P μg^{-1}chl for P solutions) to preclude the use of an average value for stationary-phase P content as an accurate correction for algal P contribution at the end of algal growth. The algal P correction procedures employed by Golterman et al. (1969), who assumed constant algal cell P content during growth, and by Sagher (1974), which was based on a constant value of stationary-phase cell content between cultures, would not provide accurate estimates of the cell P content of Anabaena grown in soil systems.

The Fe gel system evaluated here avoids the necessity of adding high levels of P to growth-control flasks in order to produce the same biomass as a particulate P source material would support. This prevents the luxury uptake of P but still allows large amounts of P to be released

slowly for algal growth, as would occur with soil P. This system allows the contribution made by algal P to soil-algal extractable P to be accurately estimated throughout the whole period of algal growth; this in turn enables the rate of depletion of soil P to be determined, rather than just the total amount of soil P depleted.

In conclusion, it appears that by manipulating the degree of P saturation of Fe gel and the amount of Fe gel used, it is possible to obtain conditions of P availability relevant to those of a wide range of soils and particulate P sources. The algal P fractionation data obtained using this system would be more applicable to the bioassay of particulate P than data obtained using P solutions which are often used in P bioassay studies.

SECTION 5

CHARACTERIZATION OF BIOLOGICALLY AVAILABLE PARTICULATE PHASE PHOSPHORUS

5.1 Introduction

Following the development of satisfactory procedures for determining algal biomass and P status in the presence of soil material (Sections 3 and 4), a combination of the bioassay technique and a soil fractionation procedure was attempted. Several workers have already evaluated the potential of using various extractants for the prediction of plant-available P (Section 2.5.2; Olsen et al., 1954; Saunder, 1956; Chang and Juo, 1963; Dalal, 1973b) and P available to algae in freshwater (Golterman et al., 1969; Sagher, 1974; Cowen and Lee, 1976). The literature suggested that an NaOH-extractable soil P fraction would probably be useful in assessing biologically-available P for a system where optimum conditions for particulate P utilization were established. Initially, 1M NaOH was evaluated as a soil extractant because it was considered that this would remove all of the biologically-available P fraction. It was considered that biologically-available P would be held at or very close to the surface of the soil particle, by sorption (implying adsorption or absorption) mechanisms. When this amount had been quantified it would be more convenient to decrease the amount of soil P extracted, by altering extraction times or concentration, so that the amount of P extracted was similar to the amount of biologically-available P.

In the preliminary experiment a simplified version of the modified Chang and Jackson (1957) soil P fractionation procedure (Williams et al., 1967) was used to characterize the forms of algal and particulate P, and to investigate which fractions of particulate P were depleted during algal growth.

The purpose of this phase of the study was to develop and evaluate an extraction procedure which would remove biologically-available P from several potential surface runoff materials.

5.2 Materials and Methods

5.2.1 Soils

Two contrasting soil types, Tokomaru silt loam (Pollok, 1975) and Okaihau gravelly clay (New Zealand Soil Bureau, 1968) were used in the preliminary study. The soils and their location are briefly described in Table 6.1. In each case the < 30-μm material, considered to be potential surface runoff material (Sharpley, 1977), was separated by dispersion of the whole topsoil (0 - 5cm) in distilled water and decantation after the appropriate settling time (Jackson, 1968). The < 30-μm material was used for all bioassay procedures in this Section and is hereafter referred to as soil or soil material, unless specific emphasis with respect to particle size is required in which case the full description is given.

5.2.2 Analytical methods

The methods used were the same as those described in Section 3, except that the nitrogen (N) analyses were carried out using a Technicon Autoanalysis system. Total Kjeldahl N was determined by the automated Kjeldahl digestion of an unfiltered aliquot of algal culture (Terry, 1966).

5.2.3 Experimental procedures

The algal culture techniques were those described in Section 3. Because the amounts of biologically-available soil P were unknown, estimates were made from the amounts of algal growth that occurred on the

soils used in Section 4. With these estimates in mind, the amount of P sorbed by the Fe gels used in the control flasks, was adjusted to give a range of solution P values close to those sustained by the soil. Mercuric chloride ($HgCl_2$. 0.05g l^{-1}) was added to non-inoculated soil cultures so that any non-biological changes in the soil P fractions could be distinguished from biological changes.

In order to determine the total amount of potentially-available soil P, all conditions were optimized for P depletion, e.g., long culture periods; P-starved inocula; and optimum light, temperature, pH and nutrient conditions for algal growth. Also, a P-assimilating alga was used and the cultures were shaken to optimize contact between the algae and particulate material, and to keep the cultures well aerated.

The sequential fractionation procedure initially adopted to chemically characterize soil P is shown in Fig. 5.1. The subsequently modified procedure incorporated a 0.1M(NaOH + NaCl) extraction at a soil: solution ratio of 1:1000 for 17hr before the 1M (NaOH + NaCl) extraction. Because NaCl is only present in the combined reagent to aid the sedimentation of fine clay particles, the extractants are hereafter referred to as 0.1M NaOH and 1M NaOH.

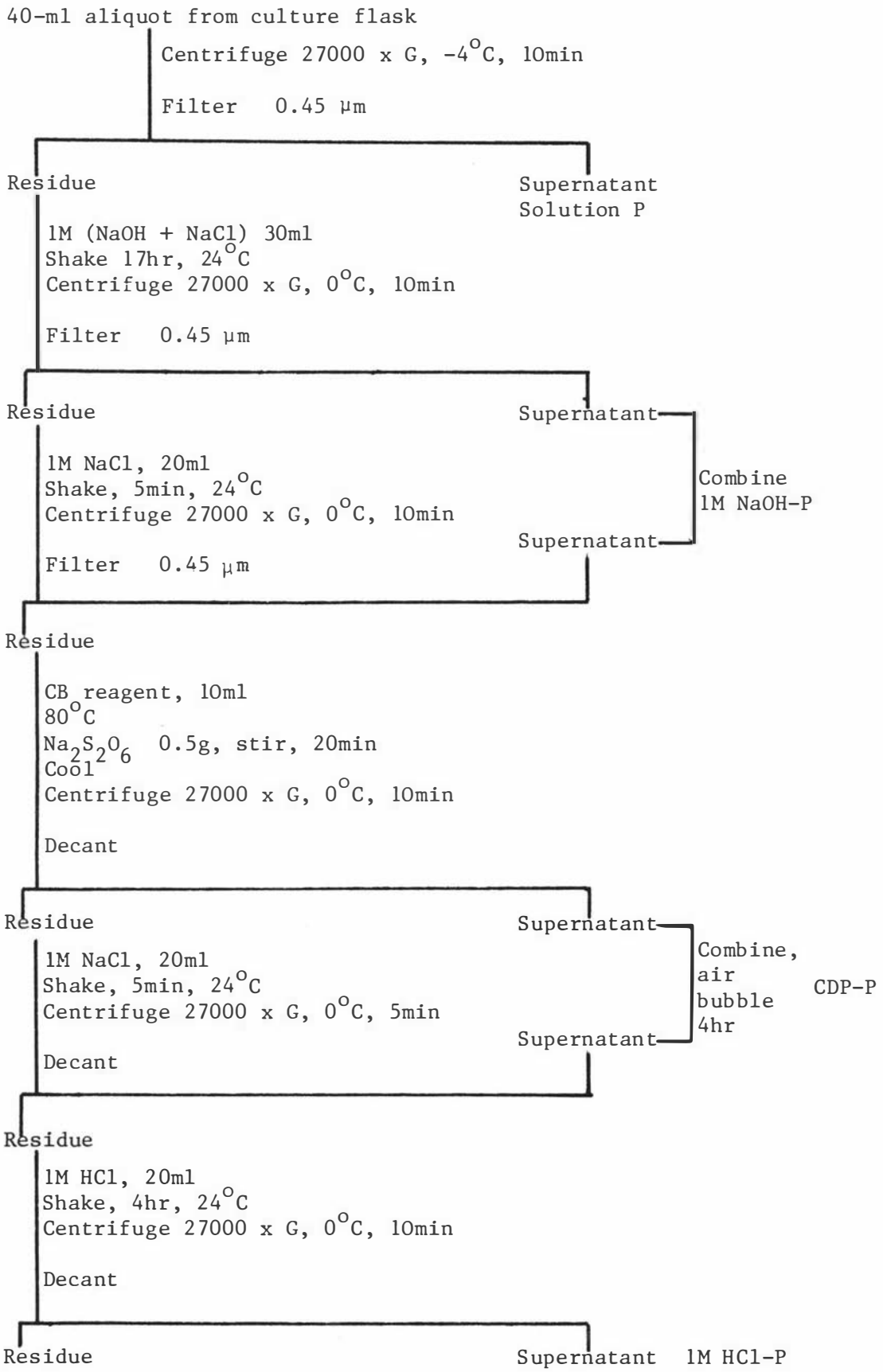
5.3 Results and Discussion

5.3.1 Combined bioassay-phosphorus fractionation procedure

5.3.1.1 Algal-growth characteristics

The bioassay procedure was continued for 29 days (Fig. 5.2), at which point the algal pigmentation in the soil culture turned yellow-green, indicating nutrient deficiency. Alkaline phosphatase activity was rapidly increasing at this point and the majority of algal cells had flocculated.

Fig. 5.1 Phosphorus fractionation procedure



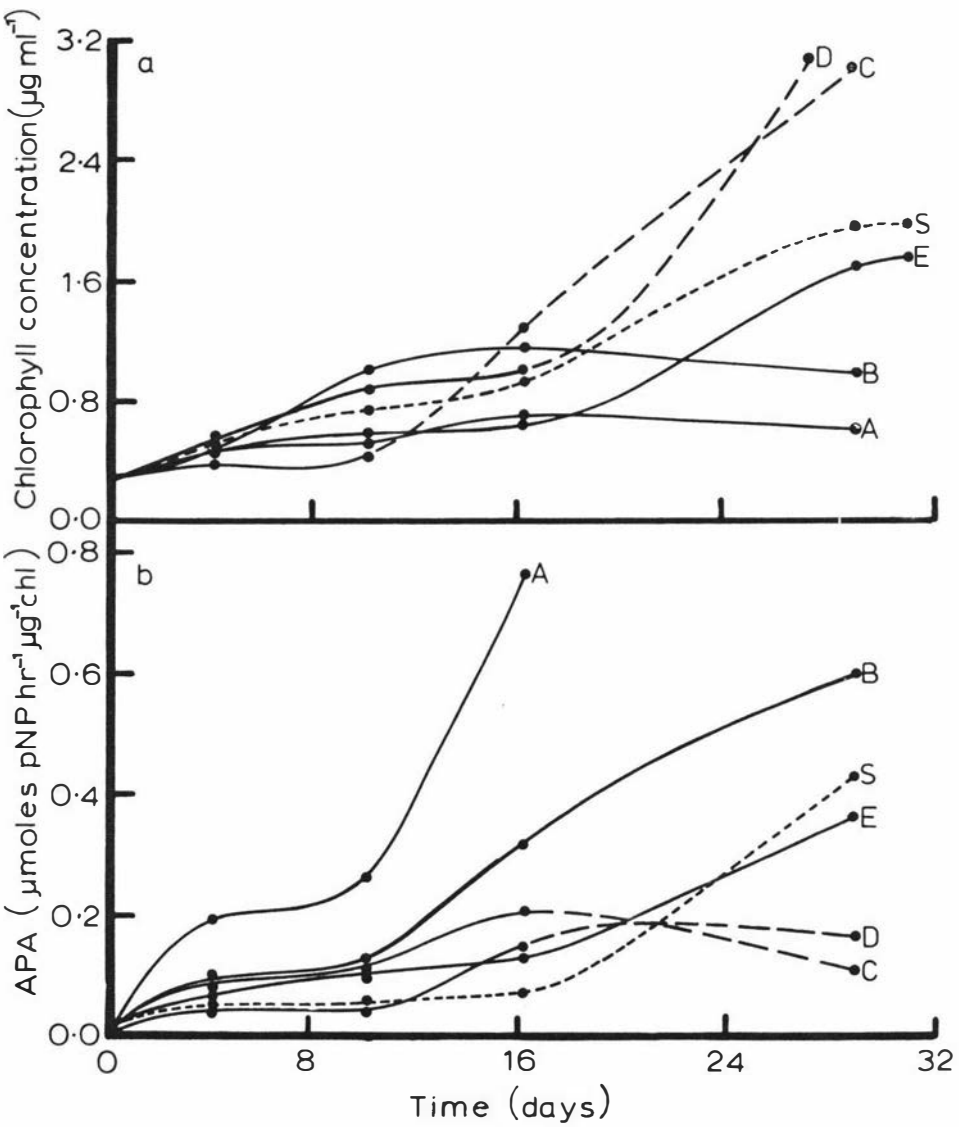


Fig. 5.2 Chlorophyll concentration (a) and alkaline phosphatase activity (APA) (b) during the growth of Anabaena on Tokomaru soil (<30 µm) (S) and on Fe gels containing increasing amounts of sorbed P (A = 0, B = 16·4, C = 18.0, D = 20.5, and E = 23.0 µg P mg^{-1} Fe gel). Dashed line for C and D after day 16 represents period after membrane rupture.

At 30 days, 500 μg P (K_2HPO_4) was added to the soil culture and to control flask E, where the Anabaena had also entered the stationary phase of growth. Algal pigmentation was restored by day 31. This indicated that algal growth had been limited solely by the absence of available P, and therefore all available soil P must have been depleted by day 29.

The Fe gel systems were used to produce the P control conditions for algal growth. The P fractionation results for algae grown in Fe gel systems showing similar P status to algae grown in soil systems, were then used to correct for algal P contribution to the soil-algae P fractionation data (Table 5.1 and 5.2). In control systems C and D (Fig. 5.2a) the dialysis memberane containing the Fe gel P source ruptured after 16 and 10 days, respectively, thereby rendering both cultures useless as sources of correction data (see later). Chlorophyll (chl) concentrations measured over the initial stages of the experiment, did not reflect well the concentration of available P in each flask (Fig. 5.2a). Although the chl measurements, except in flasks A, B, and E, were unpredictable and disappointing, the alkaline phosphatase data (Fig. 5.2b) reflected the P status of the algae extremely well, even to the extent of pin-pointing the times of rupture of the dialysis tubing in flasks C and D. The input of available P resulting from the contact between the Fe gel and the algae caused repression of the enzyme production, as observed by Healey (1974) and Keenan and Auer (1974), and a rapid increase in algal growth. The inconsistency of the chl concentrations and the slow algal growth during the first 15 days were probably caused by the inoculum being acutely P starved. A high degree of P starvation can produce more active algae for the uptake of P but if P starvation is carried too far, the cells may suffer irreversible changes. Starvation beyond this point causes cell damage

Table 5.1 Algal P fractions during the growth of Anabaena in Fe gel systems containing different amounts of sorbed P

Time (days)	Fe gel system	Amount of P extracted ($\mu g\ l^{-1}$)					
		Solution		1M NaOH		CDB-IP	HCl-IP
		IP	TP	IP	TP		
0	A(P-, gel-)	6	5	27	117	< 1	< 1
10		< 1	7	26	106	"	"
29		1	3	5	91	"	"
0	B(16.4 $\mu gP\ mg^{-1}$ gel)	2	< 1	21	180	< 1	< 1
10		< 1	8	40	209	"	"
29		< 1	< 1	27	118	"	"
0	C(18.0 $\mu gP\ mg^{-1}$ gel)	< 1	< 1	26	181	< 1	< 1
10		< 1	10	40	198	"	"
29		< 1	< 1	223[1]	746[1]	"	"
0	D(20.5 $\mu gP\ mg^{-1}$ gel)	< 1	< 1	21	180	< 1	< 1
10		< 1	3	44	371	"	"
29		< 1	< 1	717[1]	1500[1]	"	"
0	E(23.0 $\mu gP\ mg^{-1}$ gel)	2	< 1	25	180	< 1	< 1
10		1	11	28	194	"	"
29		< 1	< 1	92	275	7	"

[1] Abnormally high due to rupture of dialysis tubing.

Table 5.2 Algal and soil P fractions at various times during the bioassay of <30-μm material from Tokomaru soil

Time (days)	System	Amount of P extracted ($\mu g\ l^{-1}$)					
		Solution		1M NaOH		CDB-IP	HCl-IP
		IP	TP	IP	TP		
0	Soil ($HgCl_2$)	2	12	100	368	27	21
10		4	15	109	387	26	11
29		5	5	104	369	19	8
0	Soil-algae	2	7	155	644	32	20
10		<1	9	158	646	33	11
29		<1	<1	161	561	34	10
0	Algal contribution to soil-algae	2	-	25	194	<1	<1
10		1	11	55	228	<1	<1
29		<1	<1	107	303	7	<1
0	Corrected Soil	2	3	130	450	32	20
10		<1	<1	103	418	33	11
29		<1	<1	54	258	34	10

and results in long recovery periods (Azad and Borchardt, 1968). The 0.1M NaOH-extractable P (0.1M NaOH-P) value for the inocula was approximately 0.2 $\mu g\ P\ \mu g^{-1}$ chl, which was within the cell P limits (0.09 - 0.22 μg 0.1M NaOH-P μg^{-1} chl) in the stationary phase of growth (Section 4) where algae were showing signs of acute P starvation.

Only in culture E did the gel-grown algae have a similar P status to the soil-grown algae, as indicated by similar chl concentrations and alkaline phosphatase activities (Fig. 5.2a, 5.2b). Because only an estimate of biologically-available particulate P was required at this stage, data from culture E, although not exactly similar to the soil data, were used to correct for algal P contribution.

5.3.1.2 Algal phosphorus fractions

Anabaena was capable of reducing the concentration of both inorganic and organic solution P to below detection limits. Measurements of solution TP after day 0 (Table 5.1) were probably the result of lysis of Anabaena cells due to death or mechanical pressures caused by pipetting, centrifugation, or membrane filtration. The 1M NaOH reagent extracted significant amounts of algal P, whereas CDB and 1M HCl removed negligible amounts. Golterman et al. (1969) also found that subsequent to a 0.1M NaOH extraction of Scenedesmus cells, 0.25M H_2SO_4 did not extract any additional algal cell P. The apparent rapid decrease in 1M NaOH-algal-IP and 1M NaOH-algal-OP in flasks A and B was a direct result of algal flocculation and adherence to the flask walls, thus producing sampling errors. Flocculation was accompanied by a sudden increase in alkaline phosphatase activity and a gradual loss of pigmentation, indicating the onset of acute P starvation (Fig. 5.1a, 5.1b). The rapid increases of algal growth and in algal IP and OP (Table 5.1) in flasks C and D after

day 16 and 10, respectively, were due to the increased availability of P to the algae. Whereas the membrane ruptures were unfortunate from an experimental standpoint, the data are included to show the acceptability of alkaline phosphatase activity as an indicator of Anabaena cell P status, and the usefulness of these parameters in selecting algal data to correct for algal P in soil-algal cultures.

5.3.1.3 Soil phosphorus fractions

Solution TP constituted 3% (12 μg P g^{-1} soil) of the total extractable P from Tokomaru soil, only 2 μg P g^{-1} soil of this being IP (Table 5.2). These low values suggest that the soil had received only small fertilizer inputs. Because it was possible to monitor solution P (OP + IP) in the presence of the algae, this precluded the use of a correction procedure. Solution P, however, was reduced to below detectable concentrations. Anabaena, therefore, was able to remove P from solution at a rate which was as fast and probably faster than it could be released from the soil. Because the rate of increase of chl concentration was relatively slow in the soil culture (Fig. 5.2a), unlike the rapid increase due to available P in cultures C and D (after membrane rupture), the data indicate that soil P was strongly sorbed and only slowly released. The 1M NaOH reagent removed 85% of the soil P removed by all reagents. Within experimental error, there appeared to be little endogenous fluctuation of the IP and OP of the 1M NaOH-P fraction (Table 5.2, Soil). There was an obvious algal P contribution to the soil-algal data (Table 5.2). Thus, the data from flask E were related to the chl concentrations of the soil-algae system and algal P contributions to the extracted P fractions were calculated. Even without a correction for algal P, soil-algal TP decreased over the 29 day incubation, whereas algal TP continued to increase.

This may suggest that some soil P was converted into microbial P forms, which were not removed by the extractants used. Sampling errors due to flocculation of soil and algae may have exaggerated this decrease in TP. When a correction was made for the algal contribution, the data showed that 60% of the IP and 43% of the TP extracted from soil by 1M NaOH had been used by Anabaena. By difference, 36% of the 1M NaOH-soil OP had also been used. Certain trends in the depletion of soil P were also evident. In the first 10 days of algal growth only IP appeared to be depleted. From day 10 - 29, however, the decrease in OP constituted 70% of the P depleted. It is possibly significant that only from day 16 was there a notable increase in alkaline phosphatase activity and this or other phosphatases, produced in response to P starvation, could have been responsible for initiating the hydrolysis of soil OP. The slow depletion of soil P during the first 10 days, and the fact that only IP was depleted, add weight to the hypothesis that the algal inoculum had been severely P starved.

The CDB and 1M HCl extractions removed 6% and 5%, respectively, of extractable soil P (Table 5.2). The CDB-P fraction did not appear to decrease during the incubation of the soil-algal system. The 1M HCl-P fraction appeared to decrease by 10 μg P g^{-1} soil during 29 days. This decrease also occurred in the non-inoculated soil culture and was therefore not a biological effect. With essentially no algal P being extracted by CDB and 1M HCl (Table 5.1) no correction procedure was necessary to show that both these P fractions had little or no biological availability (Table 5.2).

5.3.1.4 Interpretations

The data indicate that solution IP and OP were biologically available. Of the 1M NaOH-soil-TP fraction, 57% remained unutilized after

algal growth had ceased. Thus, 1M NaOH-TP was a considerable over-estimate of biologically-available P. Taking into consideration the possible errors inherent in the correction procedure, it is interesting to note the significant contribution made by the OP of this soil P fraction to biologically-available P. Of the soil P used by Anabaena, 60% was 0.1M NaOH-OP. This result, in conjunction with the findings of Berman (1969) and Herbes et al. (1975), suggests that particulate OP could play an important role in aquatic primary production. Soil P removed by CDB and 1M HCl apparently was of little or no biological significance. The larger amount of 1M NaOH-OP in the corrected soil, as compared with the control soil (Table 5.2), showed an apparent under-estimation of the contribution made by algal OP and to a lesser extent IP. This suggested that in subsequent experiments care had to be taken to produce algal material which was more closely related to that grown on soils. It is therefore probable that more than the calculated 43% of the 1M NaOH-TP fraction was actually used by the algae during growth. The data suggested that if a lower concentration of NaOH or a shorter extraction time was employed, then it should be possible to extract the 43% of 1M NaOH-TP that was biologically available. Because the soil P that was depleted by Anabaena included 60% of the IP and only 36% of the OP in the 1M NaOH fraction, it was clear that the NaOH reagent employed should extract far less soil-OP than 1M NaOH.

5.3.2 Modification of phosphorus fractionation procedure

Dalal (1973b) has shown that approximately 50% of 1M NaOH-IP is extracted by 0.1M NaOH from a calcareous soil. The 0.1M NaOH-IP fraction has also given a high correlation with biologically-available P in lake sediments (Sagher, 1974) and urban runoff (Cowen and Lee, 1976). No data

were available on the amount of soil or sediment OP extracted by 0.1M NaOH, therefore a short study of 0.1M NaOH-P from the Tokomaru soil was carried out.

5.3.2.1 0.1M NaOH-extractable soil phosphorus

The < 30-μm material of Tokomaru soil was shaken with 0.1M NaOH at a soil:solution ratio of 1:750 for periods up to 17hr. The extraction of P against time curves (Fig. 5.3) indicated that there was an initial rapid release of P to 0.1M NaOH. Approximately 80% of the IP released in 16hr was released after 1hr. This suggests the near complete removal of a form, or forms of soil IP that were chemically or positionally similar. Williams and Walker (1969b) considered that 0.1M NaOH extracted non-occluded IP. The release of OP (Fig. 5.3) showed similar trends to the release of IP. The initially rapid release of OP could have resulted from OP similarly held at the soil surface. Greaves and Webley (1969) and Anderson et al. (1974) have demonstrated that OP can be sorbed by soil, probably by the same Fe and Al hydrous oxides that sorb IP. Anderson and Arlidge (1962) have shown that extraction of Fe and Al from soils reduces the capacity of a soil to sorb inositol hexaphosphate. The further (1-17hr) slow release of OP (Fig. 5.3) could have resulted from the slower dissolution of humic- and fulvic-associated organic P (Martin, 1964b; Anderson and Malcolm, 1974). Of the soil-IP extracted by 1M NaOH, 78% was extracted by 0.1M NaOH; for OP the corresponding figure was 57%.

5.3.2.2 Interpretations

Concentrations of NaOH above 0.1M extracted increased amounts of soil OP, whereas for IP only relatively small increases were obtained. Also, 1M NaOH extracted far more soil P than was biologically-available.

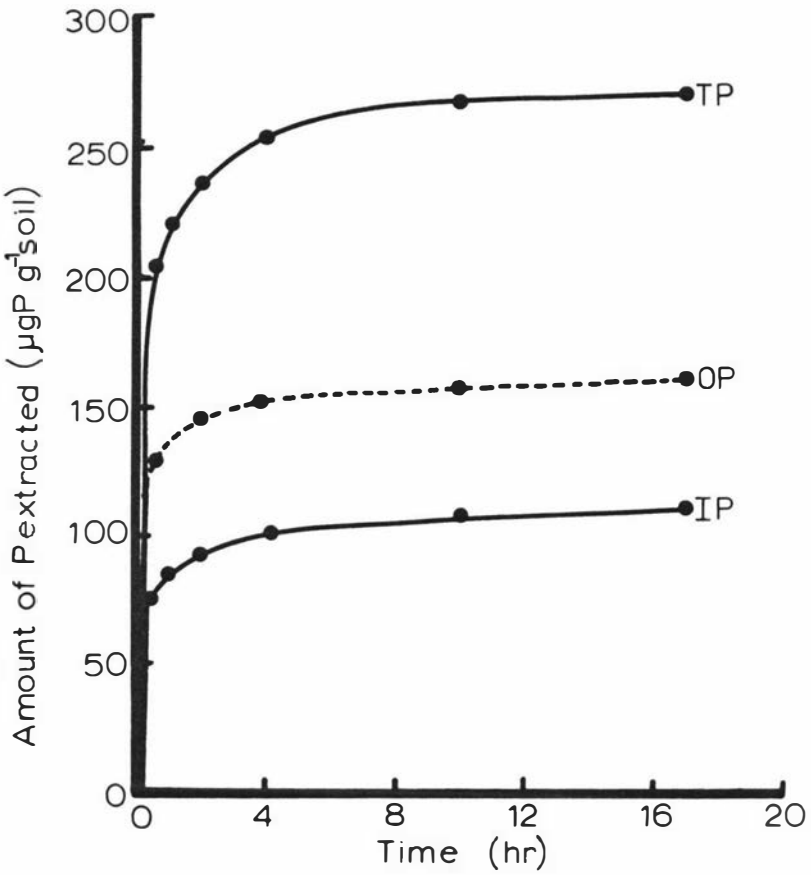


Fig. 5.3 Amounts of TP, OP, and IP extracted from Tokomaru soil (<30 μm) by 0.1M NaOH as a function of time at a soil solution ratio of 1:750.

When 0.1M NaOH was used as the extractant, the amounts of IP and OP extracted were lower. More importantly, the ratio of OP:IP extracted was also reduced. Thus, the OP which appeared to be unavailable was not extracted, whereas the IP which had shown greater availability was still extracted. The preliminary experiment showed that biologically-available P in Tokomaru soil material was approximately 60% of the IP and 36% of the OP extracted by 1M NaOH. It has now been shown that 0.1M NaOH extracted 78% of the IP and 51% of the OP extracted by 1M NaOH. Because it is considered that the correction procedure may have overestimated the algal P contribution to the soil-algal system in Section 5.3.1, the amount of biologically-available soil P may have been underestimated. Consequently, the soil P extracted from the Tokomaru soil material by 0.1M NaOH could be very similar to that P which appears to be biologically available under the conditions of the bioassay. This is evaluated further in Section 6.

5.3.3 Evaluation of the modified bioassay-phosphorus fractionation procedure

To further evaluate the chemical extractability of biologically-available soil P it was decided to repeat the fractionation procedure and to insert the 0.1M NaOH extraction before the 1M NaOH extraction (Fig. 5.1). Before incubation, care was taken to ensure that the algae were not in a condition of acute P starvation at the time of inoculation (the 0.1M NaOH-P value for the inoculum was 0.895 μg P μg^{-1} chl). It was hoped that this would reduce the lag phase and the problems that occurred in the initial study (Section 5.3.1). Also, more care was taken with the adjustment of the algal control flasks and the sampling procedures. Tokomaru soil material was again used in the study, with the contrasting Okaihau soil

which had smaller NaOH- and HCl- extractable P fractions but more CDB-P than the Tokomaru soil material (Table 6.2) and a greater ability to sorb IP. The same conditions of the bioassay were maintained as in Section 5.3.1. Total Kjeldahl N was monitored in each system to confirm that adeuqate supplies of N were being fixed by Anabaena during the incubation.

5.3.3.1 General aspects of the bioassay

The minimally P-starved inoculum produced rapid algal growth with no obvious lag phase (Fig. 5.4a). and appeared to be more able to use P from Fe gel and the soil materials, resulting in much better growth on these materials than in the previous bioassay (Section 5.3). Because of the higher initial P content of the inoculum there was also more growth in the P-, control flask (Fig. 5.5a), than with the acutely P-starved algae (Fig. 5.1a). The algal growth patterns (Fig. 5.4a) on the two soils were quite similar, as were the patterns of alkaline phosphatase activity (Fig. 5.5b). A slightly greater biomass was produced by the Tokamaru soil than the Okaihau soil. Growth on both soils ceased after 25 days (Fig. 5.5.a), when a decrease in algal pigmentation was observed. The concentration of N in all systems was still increasing (Fig. 5.6) at the point when growth had apparently become P limiting (algal 0.1M NaOH-P was 0.11 μg P μg^{-1} chl). Therefore, it was concluded that at no stage during the bioassay was N limiting algal growth. Stewart and Alexander (1971) have also shown that Anabaena is able to fix N under conditions of P starvation.

The rapid increase in enzyme activity from 0 to 5 days was not considered to indicate acute algal P starvation because the inoculum had adequate P stores and the same trend was evident in the highly-phosphated Fe gel systems 4 and 5 (Fig. 5.5b). Because the time 0 enzyme activity

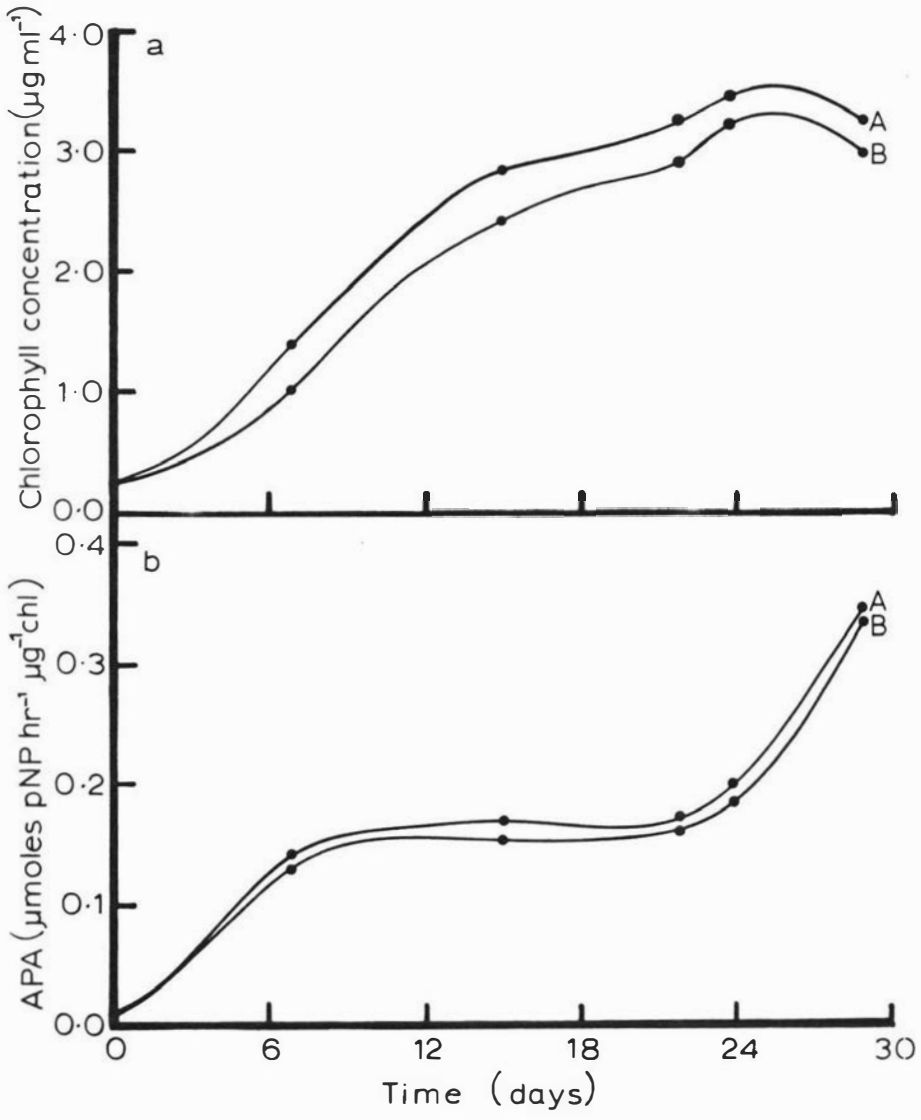


Fig. 5.4 Chlorophyll concentration (a) and alkaline phosphatase activity (APA) (b) during the growth of Anabaena on the <30-µm material from Tokomaru (A) and Okaihau (B) soils.

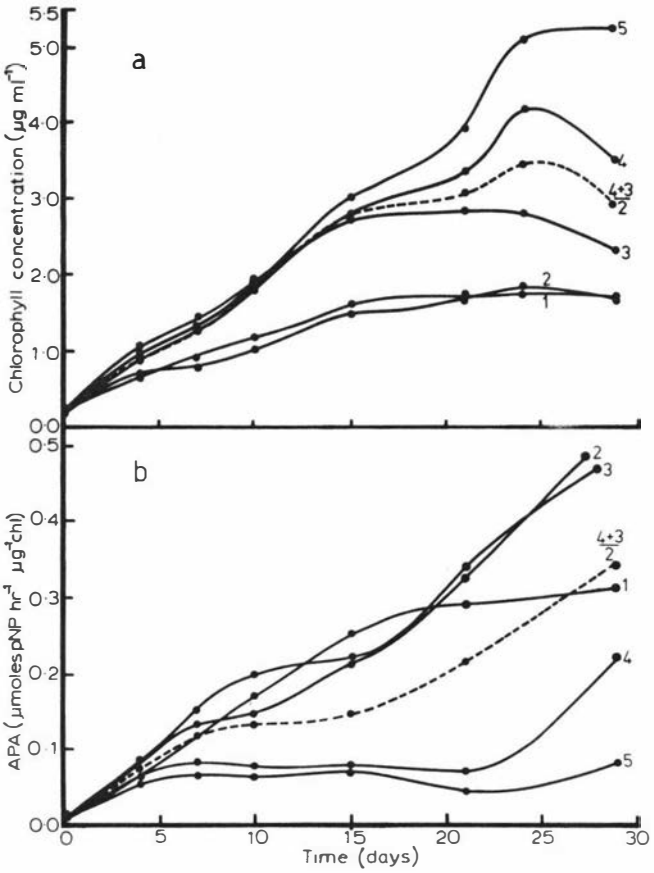


Fig. 5.5 Chlorophyll concentration (a) and alkaline phosphatase activity (APA)(b) during the growth of Anabaena on Fe gels containing increasing amounts of sorbed P (1 = 0, 2 = 20, 3 = 25, 4 = 32 and 5 = 37 µg P mg^{-1} Fe gel). Dashed line $^{4 + 3}/_{2}$ is calculated as the mean of corresponding data from systems 4 and 3.

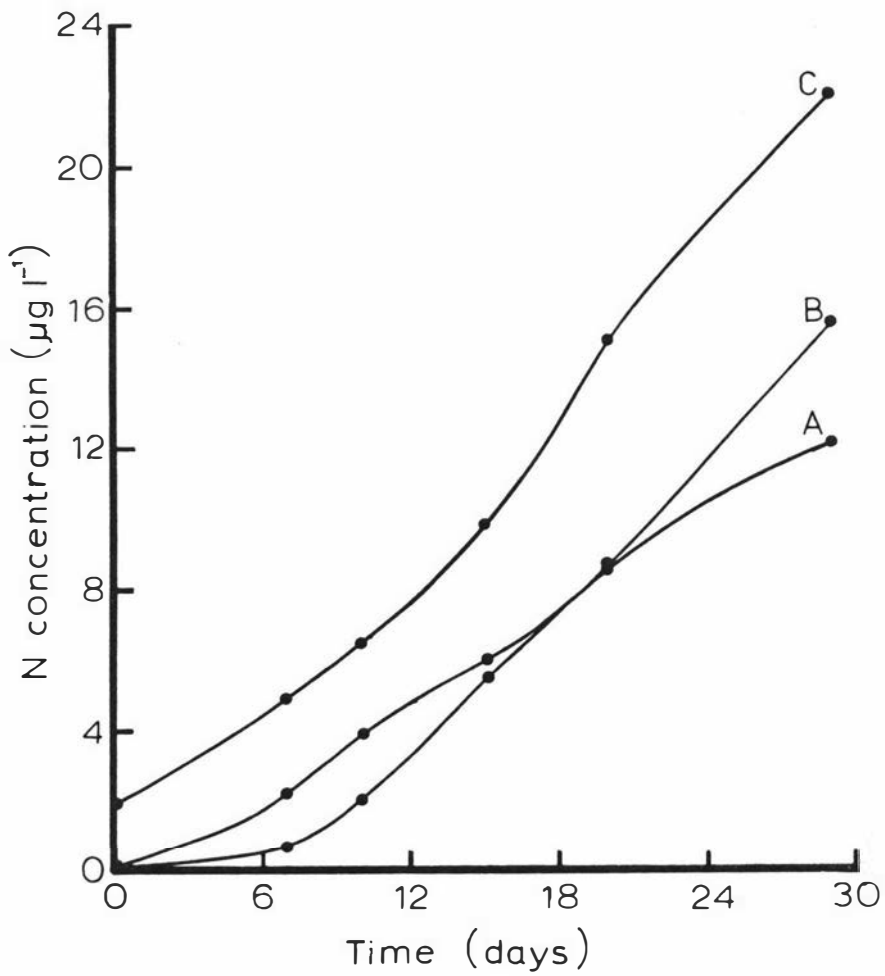


Fig. 5.6 Kjeldahl nitrogen concentration in the culture systems during the growth of Anabaena on the <30-µm material from Tokomaru (A) and Okaihau (B) soils, and in Fe gel system 4 (Fig. 5.5a) (C).

was measured immediately after inoculation of the systems, any enzyme activity would have greatly been diluted and would not be indicative of algal P status. Over the exponential growth period (day 7 to 21, Fig. 5.4a), alkaline phosphatase activity remained constant (Fig.5.4b), indicating that there was a reasonable supply of available P for Anabaena. The availability of this P source apparently decreased rapidly after 20 days, as indicated by the dramatic increase in alkaline phosphatase activity observed at 21 days (Fig. 5.4b). The algae and soil had also flocculated by 20 days.

Algal growth on the two soils (Fig. 5.4a) fell within the range produced by the Fe gel control flasks (Fig. 5.5a). When the algal chlorophyll and alkaline phosphatase levels of the soil cultures were compared with those of the control cultures, the data for both soil culture parameters fell between that of control systems 3 and 4 (Fig. 5.4 and 5.5). If the data from systems 3 and 4 were summed and the mean values calculated, it was found that the mean values closely approximated the soil values for both chl and alkaline phosphatase activity. Extractable P data were treated in the same manner and the mean data (Table 5.3) were used to correct for algal P contributions to soil-algal extractable P.

5.3.3.2 Extractable-algal phosphorus

Algal P was only extracted in significant amounts by the 0.1M and 1M NaOH reagents (Table 5.3). Solution TP only appeared during the initial stages of algal growth and rapidly decreased to below detectable levels (Table 5.4). As obtained previously (Section 5.3.1), 1M HCl and CDB removed negligible amounts of P from the algal residue. Consequently, as concluded in Section 5.3.1, the only soil-algal P fractions in which algal P constituted a major part, and for which correction was required, were the

Table 5.3 Algal P fractions calculated from the P fractionation data obtained from Fe gel systems

Time (days)	Solution	Amount of P extracted ($\mu g\ l^{-1}$)					
		0.1M NaOH		1M NaOH		CDB-IP	HCl-IP
		IP	TP	IP	TP		
0	5	53	249	31	42	< 1	< 1
7	2	70	256	-	-	< 1	< 1
10	1	77	255	34	54	< 1	< 1
15	< 1	98	280	42	72	< 1	< 1
21	< 1	111	331	41	61	< 1	< 1
29	< 1	91	360	-	67	< 1	< 1

Data obtained from systems 3 and 4 (Fig. 5.5).

Table 5.4 Solution P concentrations for soil and soil + algal systems during the bioassay of Tokomaru and Okaihau soils (< 30-μm material), and for the algal control

Time (days)	Concentration of P (μg l^{-1})[1]				
	Tokomaru		Okaihau		
	Soil[2] TP	Soil + algae TP	Soil[2] TP	Soil + algae TP	Algal control[2] TP
0	10	6	6	5.4	5
7	8	6	1	1	2
10	7	3	1	1	1
15	3	2	< 1	< 1	< 1
21	3	< 1	< 1	< 1	< 1
29	< 1	< 1	< 1	< 1	< 1

[1] As 1g of soil material used per litre, amount of P is also equal to μg g^{-1} for soil systems.

[2] $HgCl_2$ added.

two NaOH fractions. In both the 0.1M and 1M NaOH fractions, algal extractable IP and TP increased with increasing biomass but as shown in Section 4, there was no simple relationship between algal-extractable P and biomass. At inoculation, the minimally-P starved algae contributed 50% of the 0.1M NaOH-P in the soil-algal systems (Table 5.5 and 5.6). Although healthier algal growth resulted from the algae being less P starved, this large addition of algal P reduced the dependence of the algae on soil P, and therefore growth responses were partially due to the depletion of stored P. It was therefore difficult to pin-point changes in soil P availability from the chl or alkaline phosphatase data until the end of the incubation period when stored P had been depleted (Fig. 5.4). The reduced P starvation of the inoculum did not increase soil IP utilization, as was anticipated from the better growth observed. It may have been responsible, however, for the increase in the amount of soil OP depleted in this bioassay, as compared to the preliminary bioassay (Section 5.3.1). The amounts of IP depleted from the Tokomaru soil in the first (Table 5.2) and second (Table 5.5) incubations were 76 $\mu g\ P\ g^{-1}$ and 68 $\mu g\ P\ g^{-1}$, respectively, whereas the respective values for OP were 116 $\mu g\ P\ g^{-1}$ and 179 $\mu g\ P\ g^{-1}$. As mentioned previously, the first incubation value for soil OP depleted was believed to be an underestimate.

5.3.3.3 Extractable-soil phosphorus

Flocculation of the soils and algae in the systems caused greater sampling errors as the incubation experiment proceeded. It was noticeable that this produced fluctuations in the P fractionation results. All efforts were made to obtain representative aliquots, but the problems caused by flocculation, and in some cases adherence to the flask, could not be overcome. The variability seen in some results therefore, had to

Table 5.5 Amounts of 0.1M NaOH-extractable P for soil and soil + algal systems, during the bioassay of Tokomaru soil (<30-μm material) and for the algal control

Time (days)	Amount of 0.1M NaOH-extractable P ($\mu g\ l^{-1}$)[1]								
	Soil[2]		Soil + algae		Algal control[3]		Corrected soil[4]		
	IP	TP	IP	TP	IP	TP	IP	TP	OP[5]
0	93	241	125	503	54	249	71	254	183
7	142	249	127	449	71	256	56	193	137
10	105	262	128	359	79	258	49	101	52
15	129	252	153	362	104	295	49	67	18
21	157	267	154	356	111	322	43	34	- 9
29	102	218	121	368	118	360	3	8	4

[1] As 1g of soil material used per litre, amount of P is also equal to $\mu g\ g^{-1}$ for soil systems.

[2] $HgCl_2$ added.

[3] Calculated from fractionation of algae in Fe gel systems.

[4] Corrected soil = (algal + soil) - algal control.

[5] OP = (TP - IP) corrected values.

Table 5.6 Amounts of 0.1M NaOH-extractable P for soil and soil+ algal systems during the bioassay of Okaihau soil (< 30-μm material) and for the algal control

Time (days)	Amount of 0.1M NaOH-extractable P (μg l^{-1})[1]								
	Soil[2]		Soil + algae		Algal control[3]		Corrected soil[4]		
	IP	TP	IP	TP	IP	TP	IP	TP	OP[5]
0	141	242	134	427	53	249	81	178	97
7	102	260	135	428	66	255	69	173	104
10	114	249	130	376	73	256	57	120	63
15	129	208	121	342	91	267	30	75	45
21	140	254	122	354	104	295	18	59	41
29	137	253	123	375	112	337	11	38	27

[1] As 1g of soil material used per litre, amount of P is also equal to μg g^{-1} for soil systems.

[2] $HgCl_2$ added.

[3] Caclulated from fractionation of algae in Fe gel systems.

[4] Corrected soil = (algal + soil) - algal control.

[5] OP = (TP - IP) corrected values.

be accepted. The loss of fine soil particles at each decantation in the sequential fractionation, may also have been a significant source of error, given the small quantity (0.04g) of < 30-µm material used.

Only solution TP was measured because solution IP was depleted immediately after inoculation of Anabaena. Solution TP (Table 5.4) decreased to non-detectable concentrations in both the soil-algae and soil (mercuric chloride-treated) cultures of Okaihau and Tokomaru soils. Thus, part of the decrease must have been caused by resorption of dissolved OP by the soils. It was evident, however, that solution TP was initially lower and declined at a faster rate in the inoculated cultures; this indicated utilization of dissolved OP by the algae. Solution TP (5 µg P l^{-1}) was recorded in the algal control flasks immediately after inoculation. This TP could have been released by dead cells in the inoculum or by the osmotic shock caused when the old, established algal material was washed and inoculated into fresh growth media. After 10 days, all traces of solution TP had dissappeared, either because of re-assimilation by the algae or because of sorption by the Fe gel. It was noted that the initial solution TP concentration of the soil-algal system was not equal to the sum of soil solution TP plus algal solution TP, being approximately 60% of this value for the Tokomaru systems and 90% for Okaihau systems. The results suggest that a certain amount of dissolved soil OP can rapidly be assimilated by the algae. Because the P concentrations measured were extremely low, and therefore subject to error, it would be dangerous to place too much emphasis on the interpretation made.

The amounts of 0.1M NaOH-IP and -TP in the Tokomaru and Okaihau soil systems containing mercuric chloride did not vary greatly over the period of algal growth (Table 5.5 and 5.6). Even without correcting for the

algal contribution to 0.1M NaOH-P in the soil-algal systems, 0.1M NaOH-TP decreased gradually over 29 days. Extractable IP, however, did not change significantly over this period. This suggests that some of the OP in these systems was being converted to a form which was not removed from the soil-algal residue (Fig. 5.1) by 0.1M NaOH. Some of this OP may be extracted and hydrolyzed by the subsequent 1M NaOH extraction because 1M NaOH-IP, in these systems increases over the incubation period (Table 5.7 and 5.8); only a small increase was obtained for 1M NaOH-TP. When the algal contribution to the soil-algal 0.1M NaOH-P fraction (Table 5.5) was subtracted, it was found that algal P accounted for approximately 97% of the 0.1M NaOH-TP being extracted from the Tokomaru soil-algal system at 29 days. Therefore, by difference all but 3% of the 0.1M NaOH-TP fraction of Tokomaru soil had been utilized by the algae. It is clear from the results that OP made up the major part (70%) of the 0.1M NaOH-P that was depleted (Table 5.5). Although soil IP decreased slowly over the incubation period, soil OP decreased rapidly, particularly between 7 - 10 days.

At this point, alkaline phosphatase activity showed a maximum value which persisted during the exponential growth period. The increasing enzyme concentration may have been responsible for the mineralization of some OP; presumably other hydrolytic enzymes, such as phytase, could similarly have been induced, as indicated by the work of Reichardt (1971). At the end of algal growth, 0.1M NaOH-IP and -OP had been depleted by 97% and 98%, respectively.

The 0.1M NaOH-P fraction was less available in the Okaihau soil, with only 86% of the IP and 72% of the OP being depleted. Most of the OP was again utilized between 7 - 10 days, whereas the soil IP decreased slowly over the whole growth period (Table 5.6). Less OP in the Okaihau

Table 5.7 Amounts of 1M NaOH-extractable P for soil and soil + algal systems during the bioassay of Tokomaru soil (< 30-μm material) and for the algal control

Time (days)	Amount of 1M NaOH-extractable P ($\mu g\ l^{-1}$) [1]								
	Soil [2]		Soil + algae		Algal control [3]		Corrected soil [4]		
	IP	TP	IP	TP	IP	TP	IP	TP	OP [5]
0	39	104	49	191	31	50	56	141	85
7	-	-	-	-	-	-	-	-	-
10	42	196	81	200	32	55	49	155	106
15	42	147	92	217	35	59	57	158	101
21	47	109	90	185	42	61	48	124	76
29	-	150	-	201	-	62	-	139	-

1 As 1g of soil material used per litre, amount of P is also equal to $\mu g\ g^{-1}$ for soil systems.

2 $HgCl_2$ added.

3 Calculated from fractionation of algae in Fe gel systems.

4 Corrected soil = (algal + soil) - algal control.

5 OP = (TP - IP) corrected values.

Table 5.8 Amounts of 1M NaOH-extractable P for soil and soil + algal systems during the bioassay of Okaihau soil (< 30-μm material) and for the algal control

Time	Amount of 1M NaOH-extractable P ($\mu g\ l^{-1}$)[1]								
	Soil[2]		Soil + algae		Algal control[3]		Corrected soil[4]		
(days)	IP	TP	IP	TP	IP	TP	IP	TP	OP[5]
0	62	69	64	155	31	50	33	105	72
10	52	85	75	167	33	53	42	114	72
15	85	98	108	187	40	56	68	129	61
21	71	71	101	161	42	59	59	102	43
29	-	87	-	161	-	62	-	99	

1 As 1g of soil material used per litre, amount of P is also equal to $\mu g\ g^{-1}$ for soil systems.

2 $HgCl_2$ added.

3 Calculated from fractionation of algae in Fe gel systems.

4 Corrected soil = (algal + soil) - algal control.

5 OP = (TP - IP) corrected values.

soil was present in the biologically-available forms which were found in the Tokomaru soil. It may be signifcant that the 0.1M NaOH extract from the Okaihau soil contained a much larger proportion of complex humic acids (see Section 6). This is consistent with the darker colour of the extract and the larger residue of acid-insoluble material, precipitated before the measurment of 0.1M NaOH-IP, noted at this stage of the study.

Minor fluctuations in the amounts of 1M NaOH-IP and -TP were observed during the incubation of soils containing $HgCl_2$ (Table 5.7 and 5.8). There was no significant net increase or net decrease in these forms over the incubation period. When the correction was made for algal P contribution to the soil-algae 1M NaOH-P, it was evident that within experimental error 1M NaOH-IP for the Tokomaru soil remained unchanged by the growth of algae. The fluctuations in 1M NaOH-TP data made it difficult to determine whether there had been any increase or decrease in soil 1M NaOH-OP. Any such increase or decrease was of minor importance, when compared to the amounts of P depleted from the 0.1M NaOH-P fraction. The corrected data for Okaihau soil showed a slow increase in 1M NaOH-IP during the incubation. A similar increase was observed with the soil containing mercuric chloride and thus this was not an effect of algal growth. The corrected soil 1M NaOH-OP value decreased during the first 21 days. Because OP was calculated as the difference between IP and TP, and because those two results were quite variable, it was difficult to establish whether the decrease in OP was an actual algal effect or an artefact of the method. Taking the 1M NaOH-TP data as being more reliable, because they were affected by fewer procedural variables than the IP or OP data, it appears that there was no significant decrease in 1M NaOH-P from the Tokomaru soil or from the Okaihau soil over the algal-growth period.

Because algal P made a negligible contribution to CDB- or 1M HCl-P (Table 5.3), it was not necessary to use the correction procedure (Table 5.9). The amount of CDB-P for the Okaihau < 30-μm material was much lower than that reported by Williams and Walker (1969a) for the whole soil. The Okaihau (whole) soil contains coarse ironstone nodules which are presumably the major source of CDB-P (Williams and Walker, 1969b). Such nodules were not present in the < 30-μm material used in this study. The small variations observed in the CDB-P fractions of the Tokomaru and Okaihau soils, for both the soil-algal system and the system containing $HgCl_2$, are attributed to experimental error. Consequently, the CDB-P fraction in both soils had effectively no biological availability. The 1M HCl-P data also showed considerable variability for both soils. The amounts of P extracted, however, did not appear to decrease during algal growth. With the Okaihau soil it was more noticeable that a low CDB-P value was associated with a high 1M HCl-P value, and vice versa. This suggests that 1M HCl was capable of extracting occluded P which was not removed by the CDB reagent. As was the case for CDB-P, 1M HCl-P showed no biological availability.

5.3.3.4 General discussion

The rapid algal growth observed on both soils, which produced double the biomass observed in the P-control flask, clearly indicates that the < 30-μm material (potential surface runoff material) from Tokomaru and Okaihau soils could provide substantial amounts of P for algal growth. The ability of fine suspended soil material to provide P for algal growth has been observed in natural waters (Abbot, 1957; Goldman et al., 1973). Thus, it is not surprising that under optimum algal growth conditions, such a large quantity of soil P was biologically available. Cowen and

Table 5.9 Amounts of CDB- and HCl-extractable IP from soil and soil + algal systems during the bioassay of Tokomaru and Okaihau soils (< 30-μm material)

Time (days)	Amount of IP extracted (μg l^{-1})[1]							
	Tokomaru				Okaihau			
	Soil[2]		Soil + algae		Soil ($HgCl_2$)		Soil + algae	
	CDB	HCl	CDB	HCl	CDB	HCl	CDB	HCl
0	40	11	50	13	-	13	-	13
7	30	-	37	18	70	46	70	25
10	40	13	25	19	110	11	95	9
15	20	21	25	17	85	9	70	10
21	30	28	30	18	85	15	90	17

The phosphate concentration in algal extracts was < 1 μg l^{-1}.

[1] As 1g of soil material used per litre, amount of P is also equal to μg g^{-1} for soil systems.

[2] $HgCl_2$ added.

Lee (1976) estimated that 30% of the particulate IP in a fine fraction (<43 μm) of urban runoff was available for algal growth. In a study of some New Zealand soils and lake muds, McColl (1975) reported that 81% of the 0.5M H_2SO_4-extractable IP of topsoil clays could be depleted by algal growth. By selecting the fine material (< 30 μm) for bioassay in the present study, the amounts of surface-sorbed OP and IP are present in greater amounts than in a whole soil sample.

The 0.1M NaOH-P fraction, which made up approximately 50% of the total extractable P in both soils, appeared to be the only chemically-extracted P fraction which was depleted by algal growth. The subsequent fractions in the fractionation procedure showed no significant biological availability. Sagher (1974) has also shown that in Wisconsin lake sediments, 0.1M NaOH-IP was depleted by algal growth, whereas there was little or no depletion of HCl-IP. In contrast, Golterman et al. (1969) have observed HCl-P decreases in bioassays of Dutch lake muds. It is perhaps incorrect to compare the results obtained with soils to those obtained with lake sediments, because the two materials are usually very different in chemical composition. Also, soils are subject to continual nutrient loss, i.e., weathering and leaching, whereas lake sediments are sites of nutrient accumulation by deposition of detritus (this point is discussed further in Section 6.4).

The high biological availability of 0.1M NaOH-OP is somewhat surprising. For the Tokomaru and Okaihau soils, OP constituted 70% and 50%, respectively, of the soil P utilized for algal growth. Although soil OP utilization has not been studied extensively, considerable evidence points to the plant availability of OP in fine soil suspensions. Alkaline phosphatases can release IP from suspended material in lakes (Berman, 1969)

and Cooke and Williams (1973) have suggested that eroded soil OP may be hydrolyzed in a similar manner. Other workers have shown that such extracellular enzymes, which can be produced prolifically in the absence of IP, are capable of hydrolyzing various organic P compounds in the aquatic environment (Clesceri and Lee, 1965; Davis and Wilcomb, 1967; Reichardt, 1971; Herbes et al., 1975), although their effectiveness appears to decrease when OP is sorbed by the particulate phase (Philips, 1964; Greaves and Webley, 1969; Rodel et al., 1977). Only minor decreases in lake mud OP were observed by Golterman et al. (1969). The test alga (Scenedesmus obliquus) used, however, was not able to deplete solution P to below detectable levels, as was the case with Anabaena. Also the test alga was not previously P-starved to encourage alkaline phosphatase production and therefore OP mineralization. It appears that P-starved Anabaena, as used in the present study, is capable of promoting the mineralization of soil OP. In many non-eutrophic waters blue-green algae may be P-starved because P has been diagnosed as the growth-limiting nutrient (see Section 2.1; Stewart and Alexander, 1971). From this preliminary study, it can be seen that soil OP may have a substantial role to play in controlling the growth of blue-green algae in streams and lakes.

The results obtained from the incubation of the < 30-μm material from Tokomaru and Okaihau soils indicate that biologically-available P in these two soil materials can be extracted almost exclusively by 0.1M NaOH. It is considered that this reagent extracts surface-sorbed or non-occluded P (Williams and Walker, 1969b) from non-allophanic soils. It must be emphasised that all conditions were biased towards soil P depletion. Consequently, the values for biologically-available particulate P in these two soil materials should be regarded as maximum values. The

0.1M NaOH reagent could be used to assess the maximum concentrations of P which are potentially available for algal growth and present in the particulate material carried in surface runoff from, and in streams passing through, the Tokomaru and Okaihau soils. Further studies are required to assess the applicability of this procedure to other soil types.

SECTION 6

ASSESSMENT OF BIOLOGICALLY-AVAILABLE PARTICULATE PHASE P IN POTENTIAL SURFACE RUNOFF MATERIALS

6.1 Introduction

In the previous Section it was shown that for two soils, 0.1M NaOH extracted almost exclusively that P which was also available for the growth of Anabaena. In contrast, soil P from the other chemically-characterized fractions was not depleted. To determine whether biologically-available soil P was limited to the 0.1M-NaOH extractable P fraction in a range of soil materials < 30-μm material from five New Zealand soils, earthworm casts, and stream-bank material, plus surface runoff material and a river sediment were bioassayed using the combined bioassay-P fractionation procedure (Section 5). The soils were selected for their differing amounts of water-, NaOH-, CDB-, and HCl-extractable P fractions. Data published by Williams and Walker (1969a, 1969b), Syers et al. (1969), and New Zealand Soil Bureau (1968) were used to select the range of soils (Table 6.1). It was necessary to carry out three separate incubations for the shaken bioassays of the soil materials because only three materials and their respective Fe gel growth-control flasks could be accommodated in the incubator at one time. Due to the possible variation in results caused by inocula being derived from three separate algal stocks, a bioassay of all soil materials was carried out using inocula derived from the same algal stock. From the data obtained, the level of chlorophyll (chl) production was correlated with the amount of 0.1M NaOH-P originally present in each material.

Sharpley (1977) suggested that stream-bank material and particulates carried in surface runoff were the major sources of particulate P in a

Table 6.1 Soils and soil materials used in the study

Materials	Classification and parent material	Location and vegetation cover
Egmont black loam	Moderately-leached, yellow-brown loam developed from Mt. Egmont volcanic ash (andesitic)	Whareroa, Taranaki; ryegrass pasture
Atawhai steepland soil	Brown granular loam derived from massive intrusions of basaltic materials into sedimentary rocks; consequently has complex parent materials	South-east side of Sugar Loaf Hill, Nelson; rough scrub
Waimakariri silt loam	Recent alluvium developed from greywacke detritus	Waimakariri River flood plains, North Canterbury; cropping land
Waiotu brown loam	Strongly-leached brown loam developed under mixed broadleaf-podocarp or podocarp dominant forest	Waiotu, North Auckland; ryegrass pasture
Manawatu silt loam	Recent alluvium developed from greywacke detritus; has been heavily fertilized	Manawatu River levees, Te Matai Rd., Palmerston North. market gardening
Okaihau gravelly clay	Very strongly leached brown loam, with ironstone nodules, derived from argillised, olivine basalt	Okaihau, North Auckland scrub and bush
Tokomaru silt loam (Stream bank material) (Surface runoff material) (Earthworm casts)	Moderately gleyed, yellow-grey earth derived from loessial greywacke	Area studied by Sharpley (1977) Massey University: permanent pasture

stream draining a dominantly pasture catchment adjacent to Massey University. It has also been suggested that the contribution made by earthworm casts to particulate P in surface runoff P (Sharpley and Syers, 1976) could be important because earthworm casts contain proportionally more fine clay particles, which are preferentially transported in surface runoff (Ryden et al., 1973), and a higher concentration of P than the underlying soil (Barley, 1961; Gupta and Sakal, 1967; Graff, 1970; Vimmerstedt and Finney, 1973).

To assess further the applicability of using 0.1M NaOH as an extractant for biologically-available P from stream sediment source materials, the fine material from surface earthworm casts, stream-bank material (both < 30 μm), and actual surface runoff material, collected from the area studied by Sharpley (1977), were subjected to the bioassay-P fractionation procedure.

The major blooms of blue-green algae appear in streams in the warm summer months when low flow and less turbulent conditions prevail, and suspended sediment concentrations are very low. Although the shaken bioassay procedure, developed in Section 5, allows the best possible aeration and mixing of the cultures in order to determine the maximum amount of biologically-available particulate P, the extent to which the results are relevant to deposited sediment remains unclear. Chiou and Boyd (1974) have shown that muds which were able to supply P for algal growth in shaken systems were also able to supply P for algal growth in unshaken systems. Although it is appreciated that closed-culture systems do not reproduce natural aquatic conditons, it was felt that non-shaken cultures may produce conditions closer to those of the summer stream or lake environment. Consequently, in the final part of this Section, the

bioassay of all particulate materials previously studied, except earthworm-cast and stream-bank materials, was investigated in non-shaken systems where other conditions were maintained as close as possible to those of the shaken systems. The merits of 0.1M NaOH as an extractant of biologically-available particulate P are discussed with respect to both shaken and non-shaken systems.

6.2 Materials and Methods

6.2.1 Soils

The soils (Table 6.1) were selected to provide a range of different amounts and forms of particulate-phase P, as shown in Table 6.2. The potential surface runoff fraction (<30μm) was separated from the surface horizon (0 - 5cm) of each soil, as described in Section 5.2. Where necessary, < 30-μm suspensions were concentrated by centrifugation. Large volumes of surface runoff and Manawatu River sediment suspensions were concentrated by continuous centrifugation (Sorvall Superspeed RC2 B), particle-size separates were not removed and 1g of the whole sample was resuspended in ASM-1, P-, N- media (11). For all other materials except Egmont, <30-μm particle-size fractions were resuspended in ASM-1, P-, N- media (1g l^{-1}). The Egmont <30-μm material was suspended at a concentration of 0.33g l^{-1}, because of its high P content (Table 6.2), which may have produced excessive algal growth and subsequent problems resulting from overpopulation.

6.2.2 Experimental procedure

Inocula of P-starved Anabaena were produced as described in Section 3. All algal inocula were P-starved to promote uptake of P by the algae (Healey, 1973). This reduced the contribution made by algal P to soil +

Table 6.2 Amounts of P extracted from the < 30-μm material of several soils, Manawatu River sediment, earthworm casts, stream-bank material, and surface runoff material. Iron and alumium contents are included for some soils

	Amount of P extracted ($\mu g\ P\ g^{-1}$)								
	Solution	0.1M NaOH		1M NaOH		CDB-IP	HCl-IP	Fe	Al
Material	IP	IP	TP	IP	TP			%	
Egmont	10	1816	3322	-	1971	336	36	5	8[2]
Atawhai	1	123	348	81	209	540	50	8	15[1]
Waimakariri	7	115	319	33	120	198	72		
Waiotu	2	175	398	65	142	224	15	14	27[1]
Manawatu	56	754	944	180	266	290	434		
Manawatu River sediment	6	203	358	42	105	128	218		
Okaihau	3	87	210	38	60	70	15	18 11	16[1] 10[2]
Tokomaru	5	93	241	46	120	40	20	0.6[3]	
Earthworm casts	45	257	530	71	176	80	6		
Stream bank	1	84	111	30	38	114	7		
Surface runoff	4	96	218	29	99	37	5		

[1] From Williams and Walker (1969a).

[2] From Soils of New Zealand, Soil Bureau Bulletin (1968).

[3] From Hope (1978).

algal-extractable P and also reduced errors in the correction procedure. The bioassay-P fractionation procedure used to follow the depletion of soil P over the period of algal growth has been described in Section 5. Algal chl concentration and alkaline phosphatase activity were also recorded to enable corrections for the algal contribution to soil-algal P to be made. For the shaken bioassay of Waimakariri, Egmont, and Atawhai soil materials (6.3.1.1), the inocula were pre-starved to an extractable P value of 0.15µg of 0.1M NaOH-TP μg^{-1}chl. For the shaken bioassay of Manawatu River sediment and of Waiotu and Manawatu soil materials (6.3.1.2), the inocula had an extractable P value of 0.38µg of 0.1M NaOH-TP μg^{-1}chl, and the P fractionation procedure was shortened, omitting the 1M NaOH, CDB, and 1M HCl extractions because the soil P removed by these extractants had shown no biological-availability in the previously-studied materials. For the bioassay of stream-sediment source materials and earthworm-cast material (6.3.1.3), the inocula were pre-starved to an extractable P value of 0.11 µg of 0.1M NaOH-TP μg^{-1}chl.

To correlate the amounts of 0.1M NaOH-extractable P and the amounts of biologically-available P in a range of soil materials (6.3.1.4), <30-µm material from all soils, except Egmont, were bioassayed with a P-starved inoculum. The initial combined (solution TP + 0.1M NaOH-TP) concentration was measured in each flask. The concentration of chl only was monitored during algal growth.

In the bioassay study using non-shaken systems (Section 6.3.2), a 'Temperzone' plant growth cabinet was used to incubate the systems under conditions similar to the shaken studies. Before inoculation of the systems, an estimate of the amount of humic material present in each 0.1M NaOH soil extract was obtained by measuring the absorbance of the extract (HA) at 400nm.

All flasks were inoculated with P-starved Anabaena (0.10µg of 0.1M NaOH-TP μg^{-1}chl). Only solution IP and 0.1M NaOH-IP and -TP were determined during algal growth. The concentration of chl and alkaline phosphatase activity were measured at each sampling. The flasks were swirled once each day in order to minimize flocculation and clumping of soil and algae, so that uniform samples could be taken.

For each bioassay, Fe gel, P-control flasks were also incubated in order to provide data for the estimation of the algal-P contribution to soil-algal P fractions. The data used to achieve this correction are referred to at the bottom of each Table and can be found in the Tables in the Appendix. The term Correction Flask (CF) Y (where Y = A, B, C, D, or E) on Fig. 6.8 to 6.15 refers to the data obtained from the fractionation of algae growing in Fe gel system Y (Table V, Appendix), which were used to correct for the algal P contribution to 0.1M NaOH-P for that particular soil algal system.

6.3 Results and Discussion

6.3.1 Shaken bioassays of soil material

6.3.1.1 Bioassay of the < 30-µm material from Waimakariri, Atawhai, and Egmont soils

The very small amount of algal growth (chl concentration) that occurred in the P-, control flask (Fig. 6.1) during the bioassay showed that there was negligible P stored in the cells of the inoculum. The period of algal growth (11 days) was relatively short when compared to that in the bioassays in Section 5. The decreases in total algal growth and lifespan were attributed to the lower amount of stored P at the time of inoculation, because this resulted in less P being available for algal

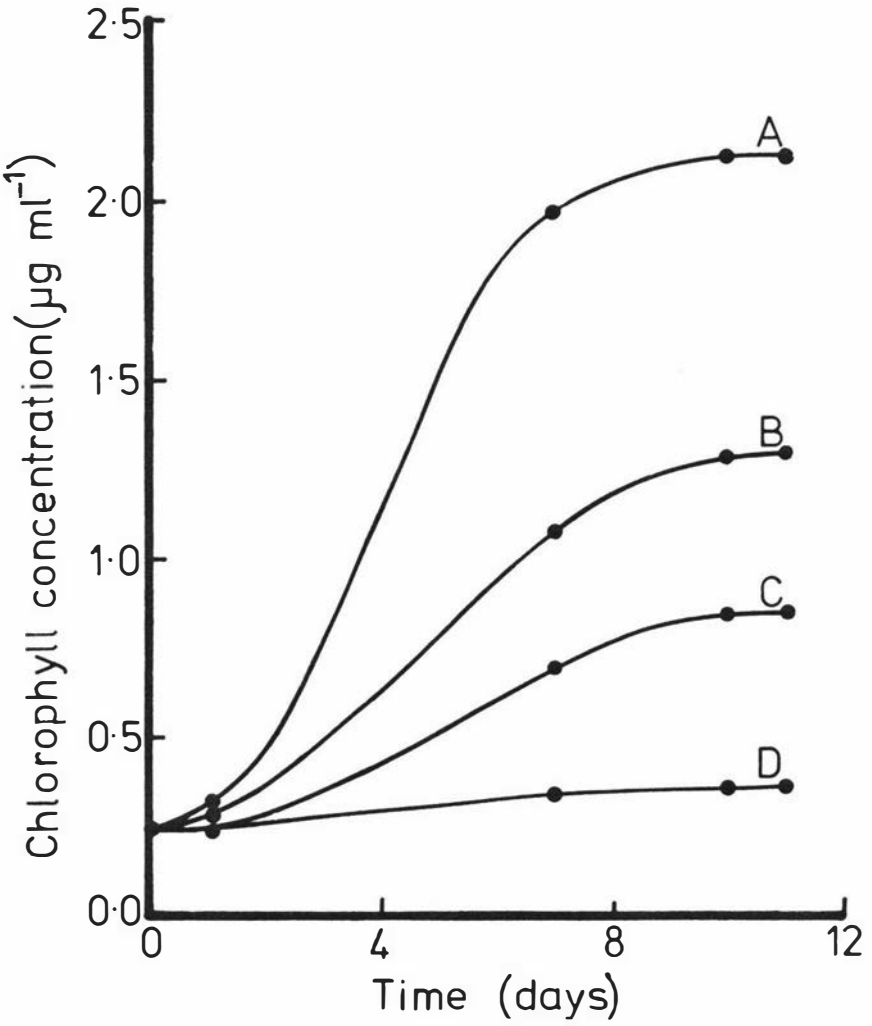


Fig. 6.1 Chlorophyll concentration during the growth of Anabaena on the <30-µm material from Egmont (A, 0.33g l^{-1}), Waimakariri (B, 1g l^{-1}) and Atawhai (C, 1g l^{-1}) soils.
D = P-, control system.

growth in each flask (i.e., the sum of algal stored P + soil-available P).

Because negliglible growth occurred in the P-, control flask all algal growth in the soil systems must have resulted from available soil P. All soils produced little algal growth over the first day of the experiment (Fig. 6.1); this was attributed to the lag-phase of algal growth. The algae were still able to assimilate P from the soil, however, as indicated by the depletion of soil 0.1M NaOH-P during this period (Table 6.3, 6.4, and 6.5). Over the first 7 days, the rapid increase in algal chl (Fig. 6.1) and the consistently low alkaline phosphatase activity (Fig. 6.2) in the flask containing Egmont soil, indicate that algal growth was not P limited and the soil P depeleted in this period had a high degree of availability. Once this form of readily-available soil P had been depleted (after 7 days), alkaline phosphatase activity increased and chl pigment rapidly deteriorated after 10 days; these changes indicated that the soil P remaining after 7 days was relatively unavailable for algal growth.

The algae growing on the Waimakariri and Atawhai soils showed more P limitation of growth, because algal alkaline phosphatase activity increased from day 1. The slow rate of increase in chl concentration and alkaline phosphatase activity reflect a gradual decrease in soil P availability in these two soils. The amount of algal chl produced by each soil (Fig. 6.1) was linearly related (μg chl produced = 0.0054 μg soil P depleted - 0.35) to the amount of available soil P (Tables 6.3, 6.4 and 6.5).

With each soil, solution IP was rapidly assimilated by Anabaena. There was also an initially rapid decrease in soil 0.1M NaOH-TP. which presumably resulted from the desorption of organic P (OP) and inorganic P

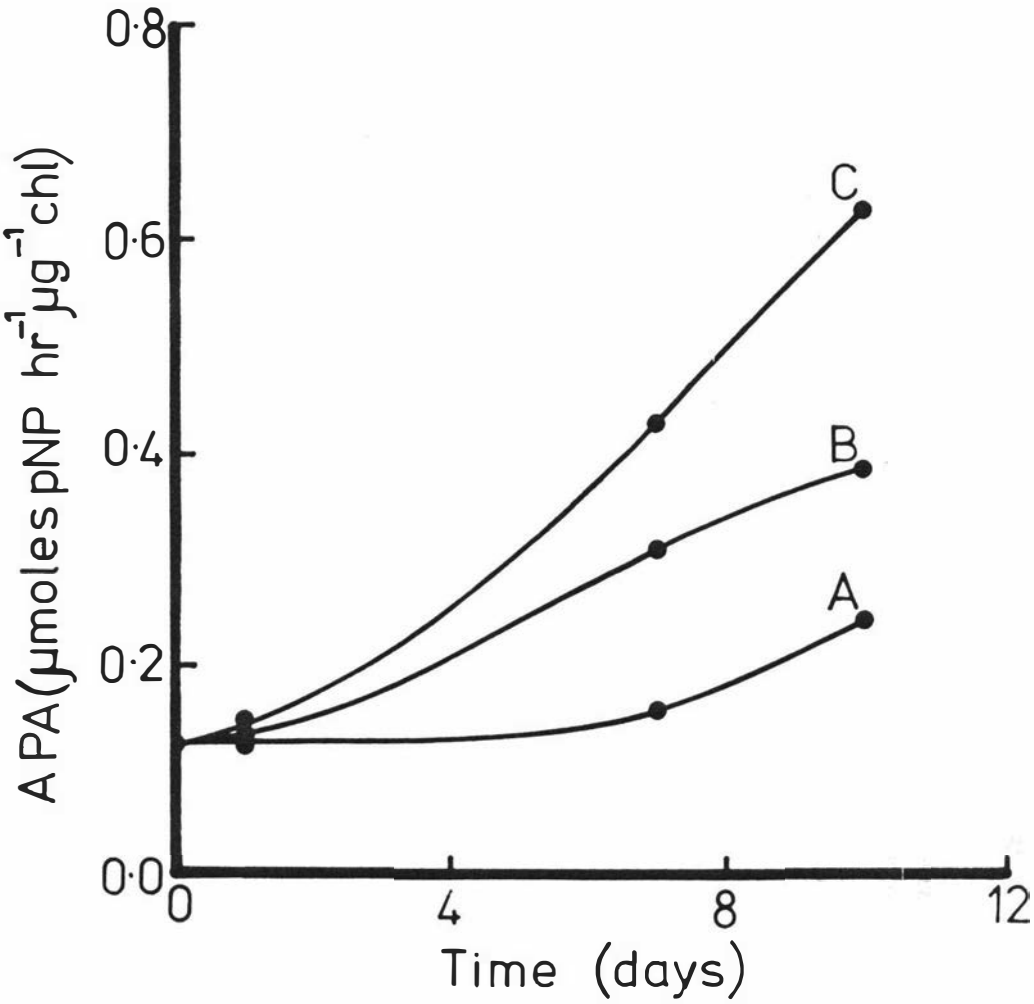


Fig. 6.2 Alkaline phosphatase activity (APA) during the growth of Anabaena on the <30-μm material from Egmont (A, 0.33g l^{-1}), Waimakariri (B, 1g l^{-1}) and Atawhai (C, 1g l^{-1}) soils.

(IP) that was weakly sorbed by the soil. The ratio of 0.1M NaOH-OP:0.1M NaOH-IP depleted from the Waimakariri and Atawhai soils during the first day of the incubation was the same as the ratio of the original amounts of 0.1M NaOH-OP:0.1M NaOH-IP present in each soil. It has been suggested (Williams and Walker, 1969b) that 0.1M NaOH removes only the surface-sorbed (adsorbed) IP from soils not containing allophane or gibbsite. Taking this into consideration and the fact that the degree of saturation of the adsorption complex would determine the amount of weakly-adsorbed P present at the surface (Ryden et al., 1977a), then the similarity of the above ratios suggests that most of the OP extracted from these two soils by 0.1M NaOH is also in the form of OP compounds adsorbed at the soil surface.

The uncorrected 0.1M NaOH-TP data for the soil + algae (Tables 6.3, 6.4, and 6.5) decreased in all soil cultures. The almost linear relationship between the amounts of chl produced in the cultures and the amounts by which the uncorrected 0.1M NaOH-TP values decreased, suggest that the increasing amounts of non-extractable TP had an algal origin; algal metabolism was presumably converting available soil P to this non-extractable form. When the algal contribution to each chemically-extracted P fraction had been subtracted (Tables 6.3, 6.4, and 6.5), it became evident that the algal growth had only depleted P from the 0.1M NaOH-P fraction of each soil. This is consistent with the results obtained with the Okaihau and Tokomaru soils in Section 5. The CDB-IP fraction was not biologically available, even in the Atawhai soil, where it constituted 50% of total-extractable P (Table 6.4b).

Algal growth on the Waimakariri soil depleted 70% of the 0.1M NaOH-soil-TP (Table 6.3a). Two thirds of the 0.1M NaOH-soil-TP was OP, and OP

Table 6.3 Chlorophyll and solution IP concentrations and algal and soil P fractions at various times during the bioassay of <30- μm material from Waimakariri soil

6.3a Chlorophyll and solution IP concentrations and data for 0.1M NaOH-extractable P

Time (days)	Chlorophyll (μg ml^{-1})	Solution IP (μg l^{-1})	0.1M NaOH-extractable P (μg l^{-1})						
			Inorganic			Organic	Total		
			Soil + algae	Algae[1]	Soil	soil[2]	Soil + algae	Algae[1]	Soil
0	0.248	12	126	15	111	225	363	37	336
1	0.319	1	103	17	86	169	300	45	255
7	1.080	1	82	22	60	76	271	135	136
10	1.290	1	69	29	40	62	257	155	102

[1] Algal P contributions determined from algal flask C data (Table II, Appendix).

[2] 0.1M NaOH (Total-Inorganic)P.

Table 6.3 contd.

6.3b Data for 1M NaOH-TP and CDB- and HCl-IP

Time (days)	Amount of P extracted ($\mu g\ l^{-1}$)						
	1M NaOH-TP			CDB-IP		HCl-IP	
	Soil + algae	Algae[1]	Soil	Soil + algae	Algae	Soil + algae	Algae
0	154	5	149	207	< 1	70	< 1
1	171	7	164	196	< 1	72	< 1
7	183	43	140	196	< 1	73	< 1

[1] Data from correction flask C (Table II, Appendix).

also made the largest contribution to biologically-available P (70%). The 0.1M NaOH-OP fraction was rapidly depleted from day 0 (Table 6.3a). Alkaline phosphatase activity gradually increased from day 0, indicating the lower availability of IP. Inorganic P comprised only 30% of the 0.1M NaOH-soil-TP and did not show the same high availability as 0.1M NaOH-OP; only 64% of 0.1M NaOH-IP was depleted by algal growth.

The considerable depletion (70%) of the 0.1M NaOH-TP fraction in the < 30-µm material from Waimakariri soil by algae suggests that the amount of soil P extracted by 0.1M NaOH would provide an approximate estimate of the amount of biologically-available P present.

Atawhai soil produced poor algal growth and the alkaline phosphatase activity, which increased rapidly from day 0, suggests that very little biologically-available P was present. Organic P and IP comprised 60% and 40%, respectively, of the 0.1M NaOH-soil-TP (Table 6.4a). The 0.1M NaOH-soil-OP and 0.1M NaOH-soil-IP were depleted by 52% and 55%, respectively during the bioassay. These two P fractions showed lower overall biological availability than the same fractions in the Waimakariri soil. Atawhai soil contained a large quantity of CDB-IP (Table 6.4b) which was not used by the algae, whereas 0.1M NaOH-IP was not significantly higher, than in other soils studied. It is possible that 0.1M NaOH removes some occluded P from this soil. The low solution IP concentration maintained by the soil implies that there is very little weakly-adsorbed surface IP. Initial algal growth was probably restricted by the lack of readily-available P and in turn a lower concentration of alkaline phosphatase was produced. Consequently, less hydrolysis of soil OP would have occurred. The smaller amount of algal biomass may have also reduced the subsequent depletion of soil IP. Only 53% of the 0.1M NaOH-soil-TP was used during

Table 6.4 Chlorophyll and solution IP concentrations and algal and soil P fractions at various times during the bioassay of <30-μm material from Atawhai soil

6.4a Chlorophyll and solution IP concentrations and data for 0.1M NaOH-extractable P

Time	Chlorophyll	Solution IP	0.1M NaOH-extractable P ($\mu g\ l^{-1}$)						
			Inorganic			Organic	Total		
(days)	($\mu g\ ml^{-1}$)	($\mu g\ l^{-1}$)	Soil + algae	Algae[1]	Soil	soil[2]	Soil + algae	Algae[1]	Soil
0	0.248	3	129	14	115	183	335	37	298
1	0.319	<1	106	16	90	150	285	45	240
7	0.692	<1	86	19	67	105	256	84	172
10	0.850	<1	74	22	52	89	243	102	141

[1] Algal P contribution determined from algal flask D data (Table II, Appendix).

[2] 0.1M NaOH (Total-Inorganic)P.

Table 6.4 contd.

6.4b Data for 1M NaOH-TP and CDB- and HCl-IP

Time (days)	Amount of P extracted ($\mu g\ l^{-1}$)						
	1M NaOH-TP			CDB-IP		HCl-IP	
	Soil + algae	Algae[1]	Soil	Soil + algae	Algae	Soil + algae	Algae
0	214	5	209	544	< 1	51	< 1
1	225	7	218	556	< 1	48	< 1
7	255	29	226	578	< 1	55	< 1

[1] Data from correction flask D (Table II, Appendix).

the bioassay. Therefore under the conditions of the bioassay, the amount of 0.1M NaOH-soil-TP was not a good estimate of biologically-available P in the < 30-μm fraction of Atawhai soil.

Unlike the soils studied previously, the larger part (57%) of the 0.1M NaOH-TP fraction from the Egmont soil was unavailable for the growth of Anabaena. In terms of the amount of P depleted, however, the Egmont soil provided much more P for algal growth than the other soils (Table 6.5a). Of the 0.1M NaOH-soil-TP remaining after algal growth, 92% was present as IP; only 15% of the 0.1M NaOH-soil-OP remained. The CDB- and 1M HCl-IP fractions were not depleted during algal growth (Table 6.5b).

Initially, the amount of 0.1M NaOH-soil-IP decreased rapidly, and this was followed by a slower rate over the remainder of the growth period. The 0.1M NaOH-soil-OP initially decreased slowly and then at a faster rate between 1 and 10 days, when alkaline phsophatase activity also increased (Fig. 6.2). The initial repression of enzyme activity (Fig. 6.2), suggests that there was an amount of readily-available IP originally present in the Egmont soil. The rapid depletion of 0.1M NaOH - soil-IP confirms this suggestion (Table 6.5a). Overall, only 27% of the 0.1M NaOH-soil-IP was available for the growth of Anabaena. This could be explained by the fact that NaOH is known to dissolve allophane (Jackson, 1968), releasing IP occluded within it (Williams and Walker, 1969b). Because of its high P content (Fig. 6.2), the allophanic Egmont is expected to contain substantial amounts of occluded P. The occluded-P almost certainly originates from native soil and fertilizer IP which has diffused into allophanic material. The 0.1M NaOH-IP fraction from Egmont soil, therefore, may be expected to contain a considerable proportion of occluded-

Table 6.5 Chlorophyll and solution IP concentrations and algal and soil P fractions at various times during the bioassay of <30-μm material from Egmont soil

6.5a Chlorophyll and solution IP concentrations and data for 0.1M NaOH-extractable P

Time	Chlorophyll	Solution IP	0.1M NaOH-extractable P ($\mu g\ l^{-1}$)						
			Inorganic			Organic	Total		
(days)	($\mu g\ ml^{-1}$)	($\mu g\ l^{-1}$)	Soil + algae	Algae[1]	Soil	soil[2]	Soil + algae	Algae[1]	Soil
0	0.248	6.6	687	19	668	255	968	45	923
1	0.275	< 1	639	27	612	244	923	67	856
7	2.006	< 1	570	43	527	85	817	205	612
10	2.140	< 1	536	48	488	40	763	235	528

[1] Algal P contribution determined from algal flask E data (Table II, Appendix).

[2] 0.1M NaOH (Total-Inorganic)P.

Table 6.5 contd.

6.5b Data for 1M NaOH-TP and CDB- and HCl-IP

Time (days)	Amount of P extracted ($\mu g\ l^{-1}$)						
	1M NaOH-TP			CDB-IP		HCl-IP	
	Soil + algae	Algae[1]	Soil	Soil + algae	Algae	Soil + algae	Algae
0	362	5	357	112	< 1	12	< 1
1	375	7	368	109	< 1	15	< 1
7	424	65	359	113	< 1	18	< 1

[1] Data from correction flask E (Table II, Appendix).

P not available to Anabaena. If only surface-bound P is available for algal growth, this may suggest that in the Egmont soil only 27% of the 0.1M NaOH-IP fraction is derived from surface-bound IP, whereas 73% is derived from occluded-IP.

The 0.1M NaOH-OP fraction from Egmont soil showed a higher availability to Anabaena than the IP fraction. The high degree of organic P availability in the Egmont soil was similar to that obtained for the Tokomaru, Okaihau (Section 5), and Waimakariri soils. The reasons why soil OP appears to show a higher availability than IP for algal growth are discussed in Section 6.4.

It is evident from the data that although the Egmont soil provides a large amount of P for algal growth, the amount of biologically-available P is overestimated by 0.1M NaOH extraction. The OP extracted by 0.1M NaOH, however, provides a close estimate of biologically-available OP present in this soil.

6.3.1.2 Bioassay of the < 30-µm material from Manawatu and Waiotu soils, and Manawatu River sediment

Initially, rapid algal growth occurred on all three materials (Fig. 6.3) and this growth, in part, resulted from P stores remaining within the inoculum. Growth in the P-, flask illustrated this point. The maximum amount of chl produced by each soil culture was linearly related to the amount of soil P depleted by algal growth (Tables 6.6, 6.7 and 6.8). This relationship (µg chl produced = 0.0065 µg soil P depleted - 0.35) was slightly different from the relationship found between the same data for the previous incubation. This indicates the necessity of using control flasks with every bioassay, so that difference in growth caused by the condition of the inoculum can be compensated for. The

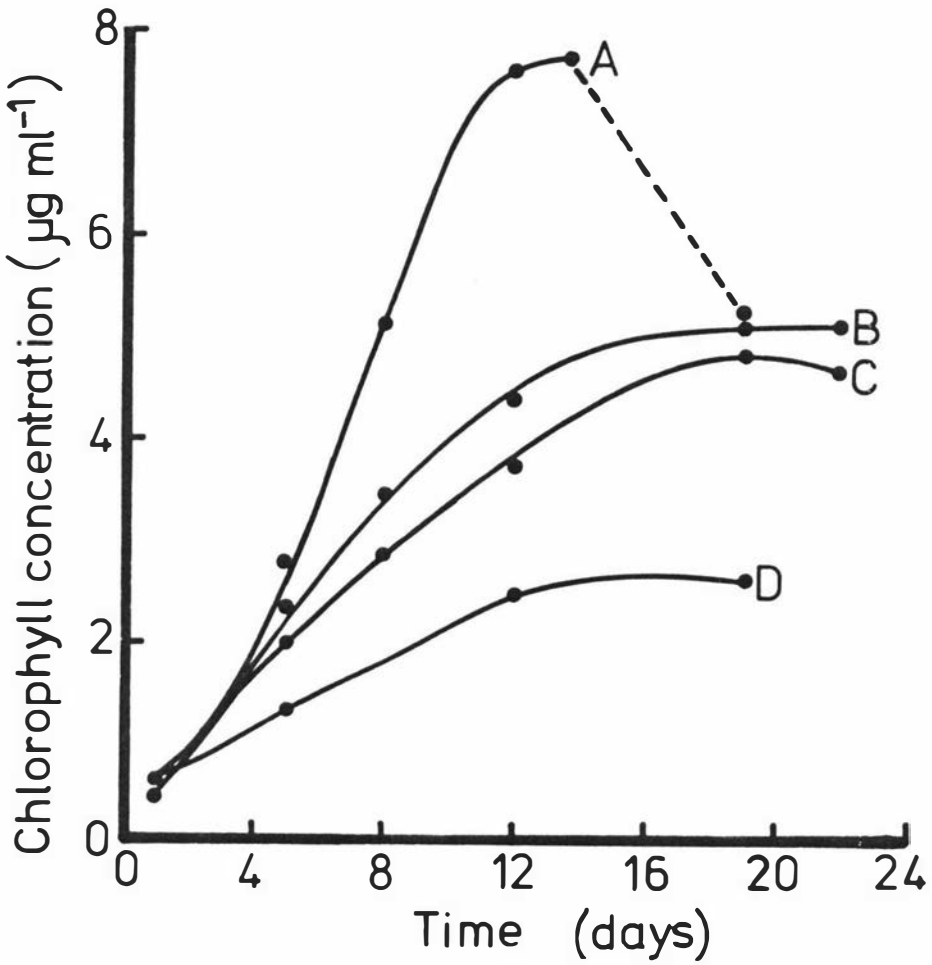


Fig. 6.3 Chlorophyll concentration during the growth of Anabaena on the <30-µm material from Manawatu (A) and Waiotu (B) soils, and Manawatu River sediment (C). D = P-, control system. Dashed line represents the deterioration of the chlorophyll pigment in system A.

repression of alkaline phosphatase activity (Fig. 6.4) indicates that the Manawatu soil was able to supply readily-available P for up to 5 days. Manawatu River sediment and the Waiotu soil did not contain similar amounts of readily-available P, because enzyme activity increased rapidly from day 1 in both cultures.

Although vigorous growth occurred in each culture, the large algal biomass produced caused sampling problems after approximately 12 days, when the algae and soil began to flocculate. At this point, growth ceased in the flasks containing Manawatu soil, whereas growth on the other two materials continued for 5 more days. Rapid deterioration of the algal pigmentation occurred in the flasks containing the Manawatu soil, which probably resulted from the high cell densities produced by exponential growth. In the Waiotu soil and the River sediment flasks, which had lower algal chl concentrations, the culture decomposition was much slower. The solution IP maintained by all three materials was rapidly assimilated by the Anabaena (Tables 6.6, 6.7, and 6.8). The extractable-soil + algal P results, when corrected for algal contribution, indicated that the 0.1M NaOH-IP and -OP fractions in all three materials were almost completely available, being 90 to 100% depeleted by algal growth. Soil IP and OP showed a higher availability in this bioassay than in the previous one. A major factor causing the increased depletion of soil P was probably the more vigorous initial algal growth resulting from P stored within the inoculum; (the inoculum had sufficient stored P to increase its biomass five-fold, Fig. 6.3).

The validity of the corrections used for algal P contributions to extractable soil-algal P could be questioned in this bioassay, because the flocculation of the soil plus algae and the algae in the control cultures had caused sampling errors. The flocculated algae in the Fe gel control

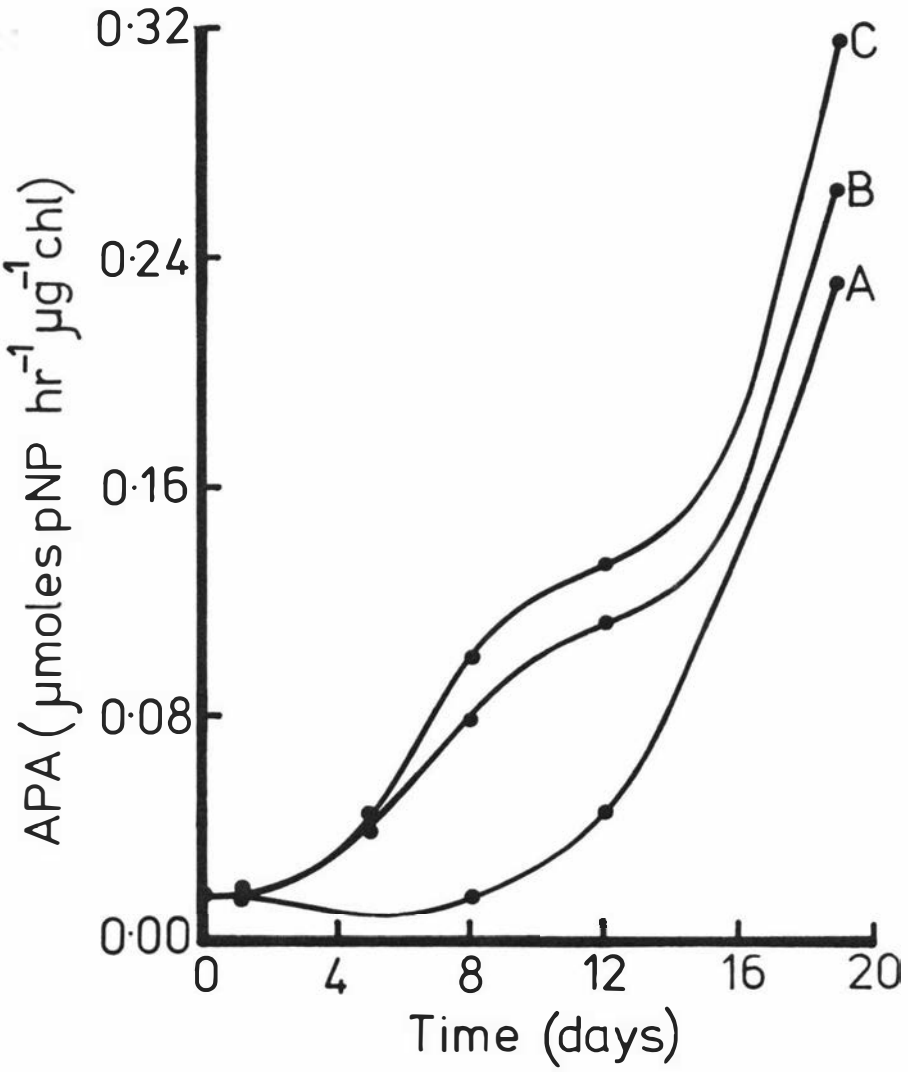


Fig. 6.4 Alkaline phosphatase activity (APA) during the growth of Anabaena on the <30-μm material from Manawatu (A) and Waiotu (B) soils, and Manawatu River sediment (C).

Table 6.6 Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of < 30-μm material from Manawatu soil

Time (days)	Chlorophyll (μg ml^{-1})	Solution IP (μg l^{-1})	0.1M NaOH-extractable P (μg l^{-1})						
			Inorganic			Organic	Total		
			Soil + algae	Algae[1]	Soil	soil	Soil + algae	Algae[1]	Soil
0	-	126[2]			754	190			944
1	0.52	61	751	101	660	140	1144	354	790
5	3.02	< 1	367	131	236	39	965	690	275
8	5.12	< 1	348	170	178	14	1057	865	192
12	7.63	< 1	286	220	66	14	1010	930	80
19	4.98	< 1	273[3]				885[3]		

1 Algal P contribution determined from flask D data (Table III, Appendix).

2 Before inoculation.

3 Sampling error due to soil-algal clumping.

Table 6.7 Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of < 30-μm material from Waiotu soil

Time	Chlorophyll	Solution IP	0.1M NaOH-extractable P (μ g l^{-1})						
			Inorganic			Organic	Total		
(days)	(μg ml^{-1})	(μg l^{-1})	Soil + algae	Algae[1]	Soil	soil	Soil + algae	Algae[1]	Soil
0		2[2]			175	223			398
1	0.48	< 1	236	78	158	230	646	258	388
5	2.63	< 1	175	70	105	102	577	370	207
8	3.31	< 1	170	90	80	- 13[3]	547	490	57
12	4.17	< 1	161	127	34	-[3]	536	510	26
19	5.08	< 1	165	141	23	-[3]	588	600	- 12
22	5.13	< 1	151	135	16	-[3]	547	557	- 10

1 Algal P contribution determined from flask C data (Table II, Appendix).

2 Before inoculation.

3 Sample errors due to soil-algal clumping.

Table 6.8 Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of Manawatu River sediment

Time	Chlorophyll	Solution IP	0.1M NaOH-extractable P ($\mu g\ l^{-1}$)						
			Inorganic			Organic	Total		
(days)	($\mu g\ ml^{-1}$)	($\mu g\ l^{-1}$)	Soil + algae	Algae[1]	Soil	soil	Soil + algae	Algae[1]	Soil
0		7[2]			203	155			358
1	0.45	<1	225	73	152	133	527	242	285
5	1.96	<1	167	53	114	43	492	335	157
8	2.80	<1	167	80	87	-[3]	473	410	63
12	2.73	<1	165	110	55	-[3]	494	515	- 19
19	4.71	<1	121	120	1	-[3]	564	561	3
22	4.65	<1	137[3]				419[3]		

1 Algal P contribution determined from flask C data (Table III, Appendix).

2 Before inoculation.

3 Sampling error due to soil-algal clumping.

systems (Table III, Appendix) had a higher ratio of 0.1M NaOH-TP:0.1M NaOH-IP during the latter stages of the bioassay, than in previous bioassays; the reasons for this are unclear. The discrepancies between calculated 0.1M NaOH-soil-IP and -TP in the Waiotu soil and Manawatu River sediment were probably the result of overestimation of 0.1M NaOH-algal-TP. If the Anabaena had reached their lowest measured 0.1M NaOH-P content (Section 4, 0.096 µg of 0.1M NaOH-TP μg^{-1} chl; Fig. 4.2b) during the stationary phase of algal growth on Waiotu soil and Manawatu River sediment, then the 0.1M NaOH-soil-TP fractions would still have been depleted by 70% and 87%, respectively. Obviously, the errors in the correction for algal P do not greatly affect the results.

The results obtained from the bioassay of the Manawatu River sediment and the <30- µm material from Manawatu and Waiotu soils indicate that under the conditions of the bioassay, 0.1M-NaOH-IP and -OP from these materials provides a close estimate of the amounts of soil=IP and -OP which are biologically available.

6.3.1.3 Bioassay of stream-sediment source materials and the <30-µm material from earthworm casts

The lack of chl production in the P-, control flask suggests that, in the flasks containing particulate materials, algal growth results only from available particulate P (Fig. 6.5). Alkaline phosphatase activity (Fig. 6.6) increased at a relatively slow rate in the flask containing earthworm casts, indicating the high availability of IP in the casts. The alkaline phosphatase data for surface-runoff and stream bank material indicate much lower particulate P availability. The lower enzyme activity in the flask containing surface-runoff material showed that the P in this material, however, was more available than that in stream-bank material.

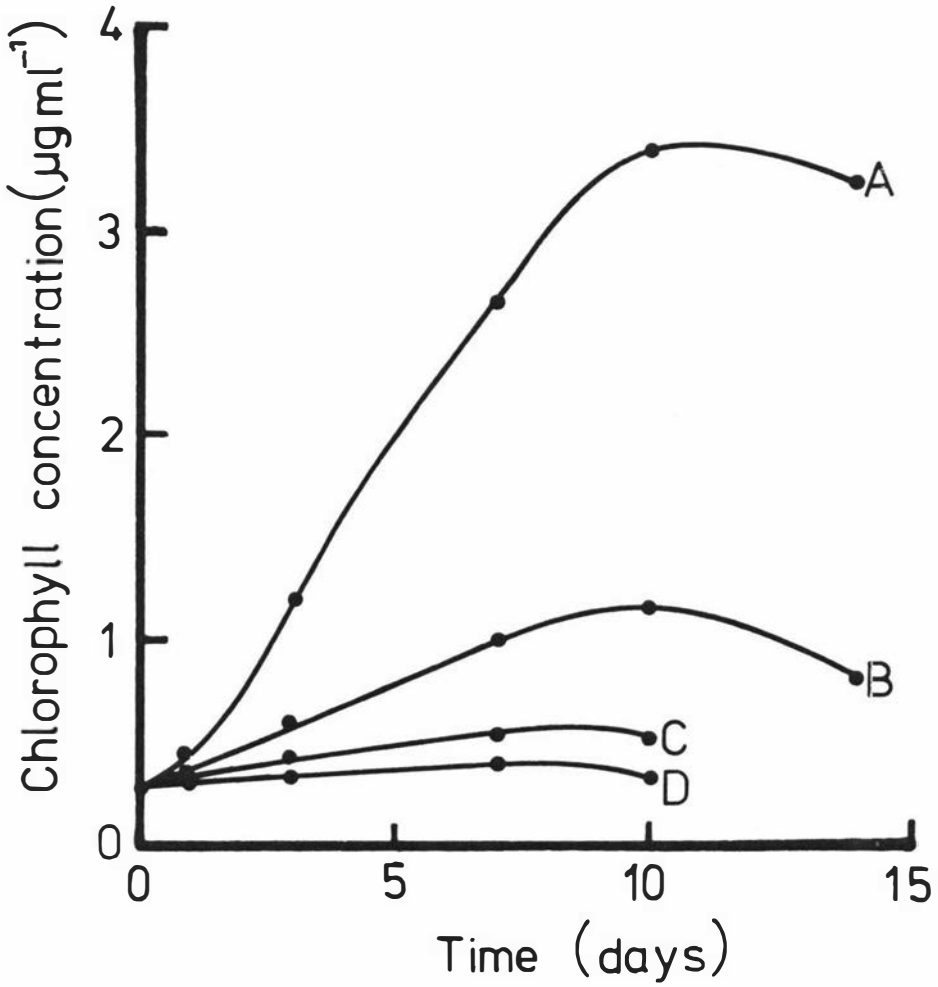


Fig. 6.5 Chlorophyll concentration during the growth of Anabaena on the <30-µm material from earthworm casts (A), surface-runoff material (B), and <30-µm stream-bank material (C). D = P-, control system.

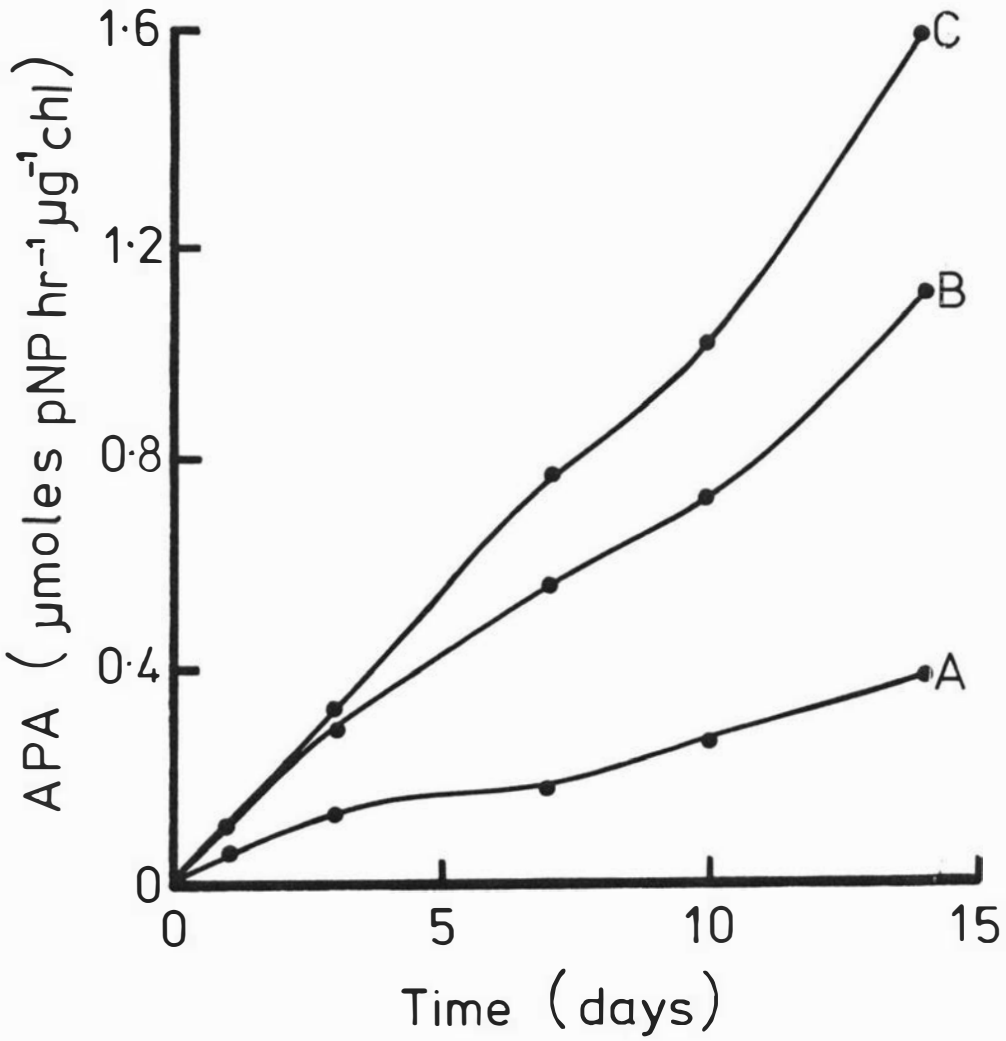


Fig. 6.6 Alkaline phosphatase activity (APA) during the growth of Anabaena on the <30-μm material from earthworm casts (A), surface-runoff material (B), and <30-μm material from stream-bank material (C).

The rates of chl production (1 to 7 days; Fig. 6.5) reflect the same trends in P availability as the alkaline phosphatase data. The maximum levels of chl recorded for each flask demonstrate that earthworm casts supplied far more P for algal growth than either surface-runoff or stream-bank material. Again, a linear relationship was obtained between the amount of soil P depleted (Tables 6.9, 6.10 and 6.11) and the maximum concentration of chl produced ($\mu g\ ml^{-1}$ chl = 0.0077 μg soil P depleted - 0.48).

Solution IP maintained by all the materials was rapidly assimilated by Anabaena (Tables 6.9, 6.10, and 6.11), including the relatively high concentration maintained by the casts (Table 6.9). When the algal P contribution to 0.1M NaOH-soil + algal - P was subtracted, it became evident that both 0.1M NaOH-OP and 0.1M NaOH-IP from casts had a high availability. Anabaena assimilated a large amount of IP from the casts during the first day of the incubation, whereas OP from the casts was not used over this period, even though OP made the larger contribution to the 0.1M NaOH-TP fraction. This may be explained in the following way. Sharpley and Syers (1976) have shown that cast material contains a large pool of loosely-bound IP, which shows a high degree of isotopic exchangeability, and which maintains a high solution IP concentration, as shown in Table 6.9. This P, which is readily assimilated by the Anabaena, would be expected to repress the synthesis of alkaline phosphatase (Fig. 6.6) and perhaps other phosphatases. This initial low concentration of hydrolytic enzymes was probably responsible for the slow utilization of cast-OP at the beginning of the bioassay. The vigorous algal growth produced by this readily-available form of P was able to deplete 0.1M NaOH-cast-OP during the remaining period of the bioassay. Overall, 79% of the 0.1M NaOH-TP, from earthworm casts, was depleted during the period of algal

Table 6.9 Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of <30-μm material from earthworm casts

Time (days)	Chlorophyll ($\mu g\ ml^{-1}$)	Solution IP ($\mu g\ l^{-1}$)	0.1M NaOH-extractable P ($\mu g\ l^{-1}$)						
			Inorganic			Organic	Total		
			Soil + algae	Algae[1]	Soil	soil	Soil + algae	Algae[1]	Soil
0	0.26	45[2]			257	273			530
1	0.32	< 1	203	33	170	267	547	110	437
3	1.21	< 1	197	82	115	146	539	278	261
7	2.65	< 1	194	142	52	-	-	-	-
10	3.38	-	-	-	-	-	-	-	-
14	3.26	< 1	203	171	32	84	542	426	116

[1] Algal P contribution determined from flask D data (Table IV, Appendix).

[2] Before inoculation.

growth; this indicates that the amount of biologically-available P in earthworm casts and 0.1M NaOH-TP in earthworm casts is very similar.

A high degree of availability (86%) was also shown by the 0.1M NaOH-TP fraction of surface-runoff material (Table 6.10). The major part of this fraction was OP, which was depleted to a greater extent (95%) than the extractable IP (75%). The rate of depletion of the 0.1M NaOH-IP fraction was rapid over the first day, followed by a much slower rate of depletion over the remainder of the bioassay. The depletion of the 0.1M NaOH-OP fraction showed similar trends, except that the rate of depletion of OP increased in the latter stages of the bioassay; this was attributed to the high activity and concentration of alkaline phosphatase measured at this time, (Fig. 6.6). The almost complete utilization of the 0.1M NaOH-OP fraction, which was not observed with the earthworm casts (Table 6.9), was attributed to the presence of higher enzyme activity throughout the bioassay of surface-runoff material. The availability of the 0.1M NaOH-TP fraction (75%, Table 6.10) was lower in the actual surface-runoff material from Tokomaru silt loam, than the potential runoff fraction (< 30-µm material) from the surface soil (96%, Table 5.4). Only approximately 90% of the particuates in the actual surface-runoff material are below 30 µm in diameter (Sharpley, 1977). Consequently the smaller average particle size in the potential runoff fraction (< 30µm) would have a larger surface area and this may have resulted in a greater depletion of P. The minimally-P starved inoculum used in Section 5.3.3 produced more vigorous growth, which would also have promoted the depletion of soil-P.

The similarity in the amounts of biologically-available P in actual surface-runoff material from Tokomaru silt loam and the simulated (< 30µm) surface-runoff material separated from the surface soil suggests that the

Table 6.10 Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of surface-runoff material

Time (days)	Chlorophyll ($\mu g\ ml^{-1}$)	Solution IP ($\mu g\ l^{-1}$)	0.1M NaOH-extractable P ($\mu g\ l^{-1}$)						
			Inorganic			Organic	Total		
			Soil + algae	Algae[1]	Soil	soil	Soil + algae	Algae[1]	Soil
0	0.26	4[2]			93	111			204
1	0.42	< 1	84	16	68	76	201	57	144
3	0.58	< 1	75	22	53	51	194	90	104
7	1.01	< 1	64	41	23	5	179	151	28
10	1.15								
14	0.79	< 1							

[1] Algal P contribution determined from flask B data (Table IV, Appendix).

[2] Before inoculation.

latter provides a reasonable simulation of the former.

The stream-bank material showed low P availability; only 57% of the 0.1M NaOH-TP fraction was depleted during algal growth (Table 6.11). As expected, OP was only a minor part of the 0.1M NaOH-TP fraction in this subsoil material. Immediately after the inoculation of the algae, the 0.1M NaOH-soil-OP fraction increased. This was the result of algal OP being underestimated, the algal TP content in the stream-bank flask being higher than the algal-TP content in the control flask. The low solution P maintained by the stream-bank material (Table 6.11), the low amount of 0.1M NaOH-IP, and the high amount of CDB-IP (Table 6.2) all indicate that stream-bank material contained a small amount of weakly-adsorbed P. It follows that any surface-sorbed P would be strongly held. The lack of readily-available P probably resulted in the poor initial algal growth observed on the stream-bank material. In turn, the poor algal growth would have resulted in the decreased utilization of the more strongly-sorbed portion of the 0.1M NaOH-soil-P fraction. Other workers have reported on the low extractability of P in stream-bank material which, being subsoil material, often has a high P sorption capacity (Taylor and Kunishi, 1971; Sharpley, 1977) capable of reducing solution P concentrations to low levels in an aquatic environment. The present study indicates that although the quantity of biologically-available P in stream-bank material may be small, eroded stream-bank material is still capable of supporting algal growth under conditions favouring the utilization of particulate P. Biologically-available P in stream-bank material was considerably over-estimated by the amount of P extracted by 0.1M NaOH. This may be expected because the amounts of weakly-absorbed IP would be much lower in subsoil than in surface-soil material and thus the amount of readily-available P

Table 6.11 Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of the < 30-μm material from stream- bank material

Time	Chlorophyll	Solution IP	0.1M NaOH-extractable P ($\mu g\ l^{-1}$)						
			Inorganic			Organic	Total		
(days)	($\mu g\ ml^{-1}$)	($\mu g\ l^{-1}$)	Soil + algae	Algae[1]	Soil	soil	Soil + algae	Algae[1]	Soil
0	0.26	i[2]			83	24			107
1	0.31	<1	80	12	68	45	147	34	113
3	0.40	<1	52	22	30	20	105	51	50
7	0.50	<1	55	24	31	16	99	52	47

[1] Algal P contribution determined from flask A data (Table IV, Appendix).

[2] Before inoculation.

present in the 0.1M NaOH-P fraction would be smaller. This would result in an apparent decreased utilization of 0.1M NaOH-P. In fact the residual amount of 0.1M NaOH-soil-P remaining after algal growth was only slightly greater (Table 6.11) than in the surface runoff material (Table 6.10). For the materials containing substantial amounts of weakly-sorbed P (earthworm casts and surface-runoff material), biologically-available P could be characterized by 0.1M-NaOH extraction.

The results clearly demonstrate that both surface-runoff and stream-bank materials can act as sources of biologically-available P under conditionsfavouring particulate-phase P depletion. Earthworm casts appear to contain a relatively large amount of biologically-available P. Their susceptibility to transport in surface-runoff (Sharpley and Syers, 1976) suggests that they may be an important source of available particulate-phase P entering streams in areas which have significant quantities of surface-runoff and where surface-casting earthworms are active.

6.3.1.4 Correlation between the amounts of biologically-available P and 0.1M NaOH-extractable P in a range of soil materials

The 0.1M NaOH reagent appeared to extract, almost exclusively, biologically-available P from the Tokomaru, Okaihau, Waimakariri, Waiotu, and Manawatu < 30-μm materials, and from earthworm-cast material, surface-runoff material, and Manawatu River sediment, but not from the Egmont and Atawhai < 30-μm materials nor from stream-bank material. The extent to which variations in the biological-availability of the 0.1M NaOH-P fraction were due to the varying physiological condition of the inocula is uncertain. It was difficult to produce identical inocula for each bioassay.

To obtain an overall correlation between the amount of 0.1M NaOH-P

and P available for algal growth; it was therefore necessary to incubate all soils with algal cells derived from the same inoculum, in order to minimize the other variables. Only 15 flasks could be placed in the reciprocating incubation at any one time, which did not allow Fe gel, algal-growth control flasks to be incubated simultaneously. The exact fraction of soil P depleted could not therefore be determined, but the maximum amount of chl produced in a particular soil system could be related to the amount of 0.1M NaOH-soil-TP originally present.

The 0.1M NaOH-TP values for the soil suspensions were corrected for the amount of soil removed for analysis to give a value for the total amount of 0.1M NaOH-TP present in each flask (Table 6.12). After monitoring chl concentration during algal growth, the maximum chl concentration attained in each culture was plotted against the original amount of Solution TP + 0.1M NaOH-TP present in each flask (Fig. 6.7). A correlation coefficient of $r = 0.989$ was obtained for the relationship in Fig. 6.7. Chlorophyll production was, therefore, linearly related to the amount of Solution TP + 0.1M NaOH-TP for all the < 30-μm materials evaluated. When data for the materials not studied in this bioassay, but in previous bioassays, were included in Fig. 6.7 (stream-bank material, Manawatu River sediment, and earthworm casts), the same relationship was obtained.

Because it has been shown that chl production in a closed system is linearly related to the amount of available P present (Section 2), it may be assumed that for shaken, incubated bioassays, the amount of Solution - TP + 0.1M NaOH-TP is very closely related to the actual amount of biologically-available P present in the materials evaluated. The high correlation obtained confirms the earlier findings with shaken bioassays, which used separate inocula, where the majority of the 0.1M NaOH-TP from

Table 6.12 Maximum chlorophyll concentration and amount of 0.1M NaOH-extractable P[1] in a bioassay of <30-μm material from a range of soils

Material	Maximum chlorophyll (μg ml^{-1})	Solution TP (μg l^{-1}) (A)	Amount of TP extracted (μg g^{-1})		
			0.1M NaOH		
			Initially in flask (B)	Removed for analysis (C)	(A + B)[1] - C
Manawatu	6.87	135	756	64	827
Tokomaru	1.22	12	235	18	229
Atawhai	1.50	4	344	24	324
Waimakariri	1.66	10	309	22	297
Okaihau	1.23	2	210	15	197
Waiotu	2.40	5	439	32	412

[1] Solution TP included in 0.1M NaOH-extractable TP.

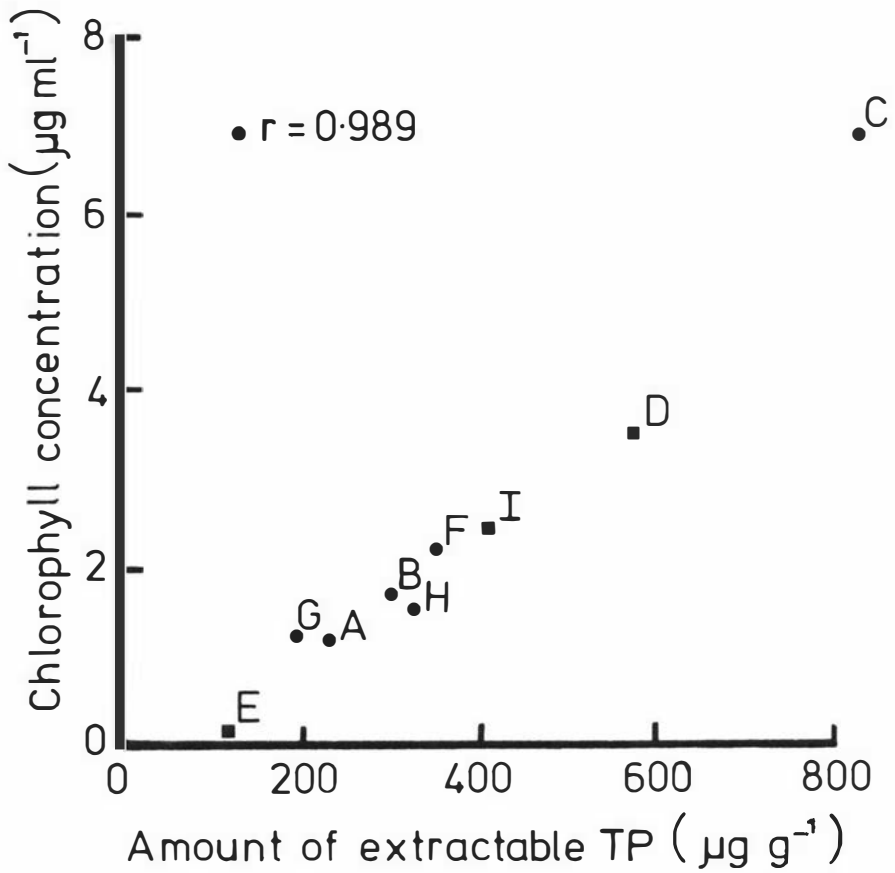


Fig. 6.7 Relationship between maximum chlorophyll concentration produced during the growth of Anabaena on a range of soil materials (<30 µm) and the amount of extractable TP in each system (given by the sum of solution-TP and 0.1M NaOH-soil-TP before algal growth).
A = Tokomaru, B = Waimakariri, C = Manawatu, D = earthworm casts, E = stream-bank, F = Manawatu River sediment, G = Okaihau, H = Atawhai, and I = Waiotu. Data for C, E, and I were obtained from a previous bioassay and were not used for the calculation of the correlation coefficient (r).

most of the materials studied was used for algal growth.

6.3.2 Non-shaken bioassay of <30- μm material from all source materials

During this bioassay, flocculation of algae and clumping of soil and algae were more noticeable than in the shaken studies. The algae no longer remained planktonic but were associated with the sediment layer at the bottom of each flask. There was no apparent relationship between the absorbance (HA) of a 0.1M-NaOH extract and the amount of OP in that extract (Fig. 6.8 to 6.15). If the absorbance reading is a valid measure of humic content, then it may be assumed that most of the 0.1M NaOH-OP from the soil materials is not associated with humic material.

The final amounts of chl (Fig. 6.8 to 6.15) produced on each soil material and in the Fe gel, P control flasks were lower than those produced by the similar shaken studies (Section 6.3.1) and the corresponding growth rates were also slower. The 0.1M NaOH-algal-P data (Table V Appendix) showed that Anabaena had assimilated large amounts of P from the Fe-gel systems, even though this did not result in high chl concentrations. The reasons for the lack of algal growth are not clear. The only difference in the conditions between this and previous bioassays was the suspension of the algae and soil by shaking. Consequently, it can be assumed that shaking induced greater algal growth by better aeration of the culture, mechanically aiding the separation of growing algal filaments, or by allowing more efficient use of the light source. The low amount of algal growth which resulted in these unshaken flasks was not P limited because the Anabaena had high amounts of extractable cell P (Table V Appendix). The alkaline phosphatase activities recorded for the soil cultures over the period of algal growth were slightly lower than in the shaken systems.

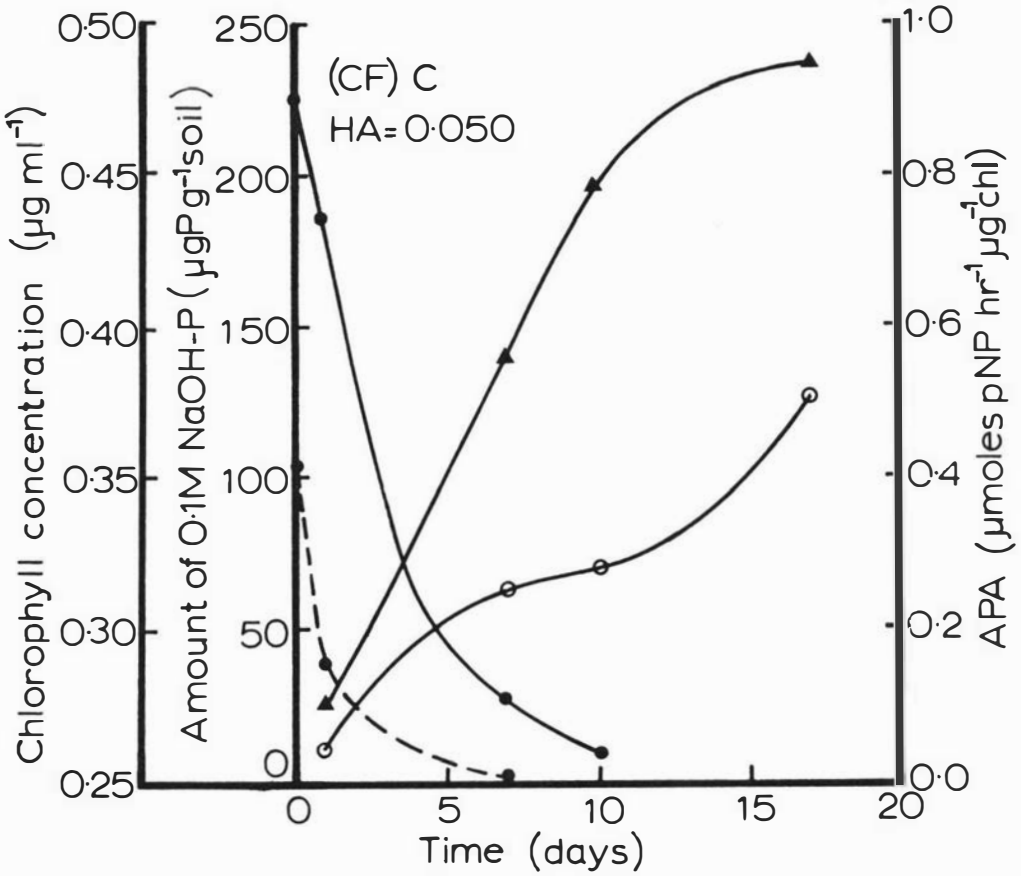


Fig. 6.8 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●—●), and alkaline phosphatase activity (APA) (—⊖—) during the growth of Anabaena in the non-shaken bioassay of Tokomaru soil (<30 µm).

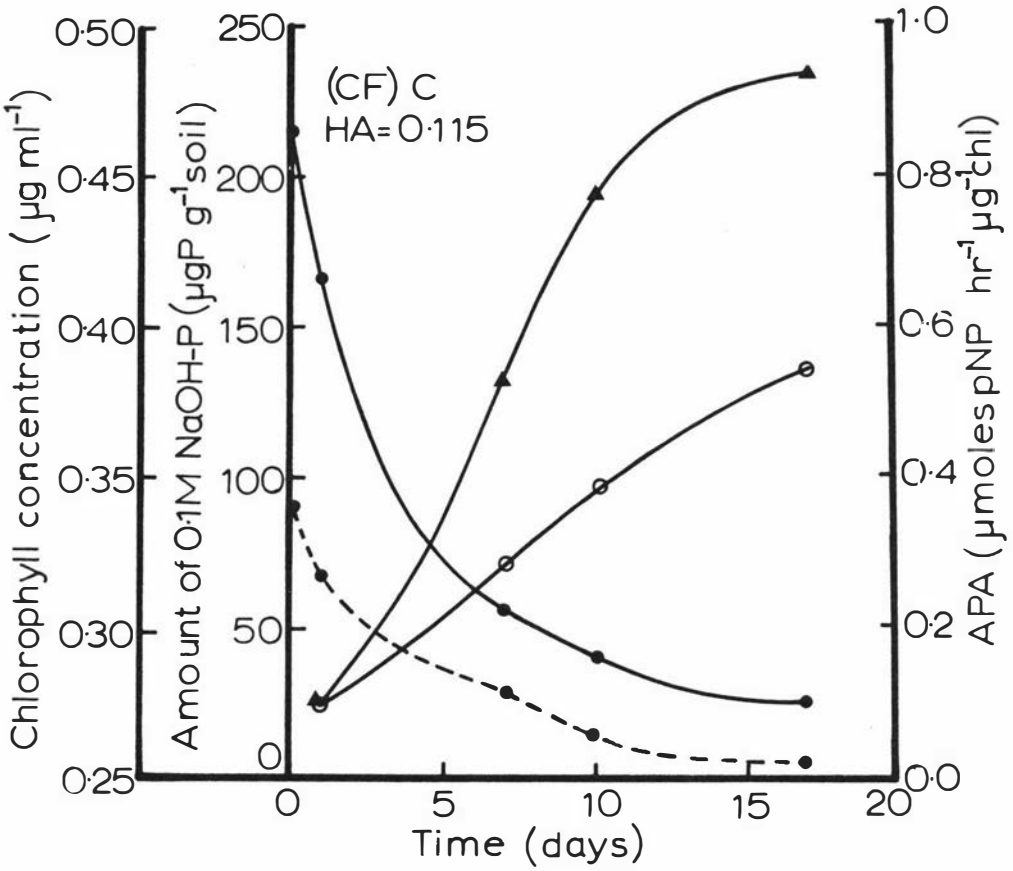


Fig. 6.9 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●—●), and alkaline phosphatase activity (APA) (—⊙—) during the growth of Anabaena in the non-shaken bioassay of Okaihau soil (<30 µm).

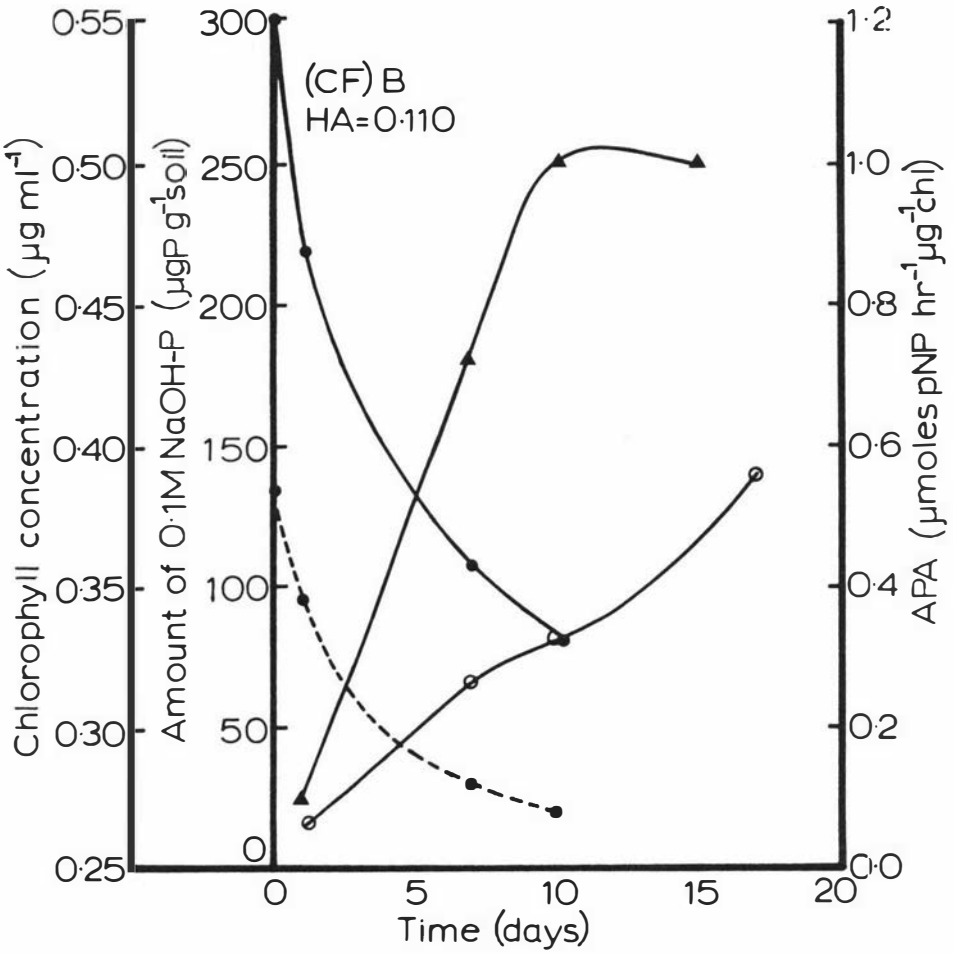


Fig. 6.10 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●——●), and alkaline phosphatase activity (APA) (—⊖—) during the growth of Anabaena in the non-shaken bioassay of Waiotu soil (<30 µm).

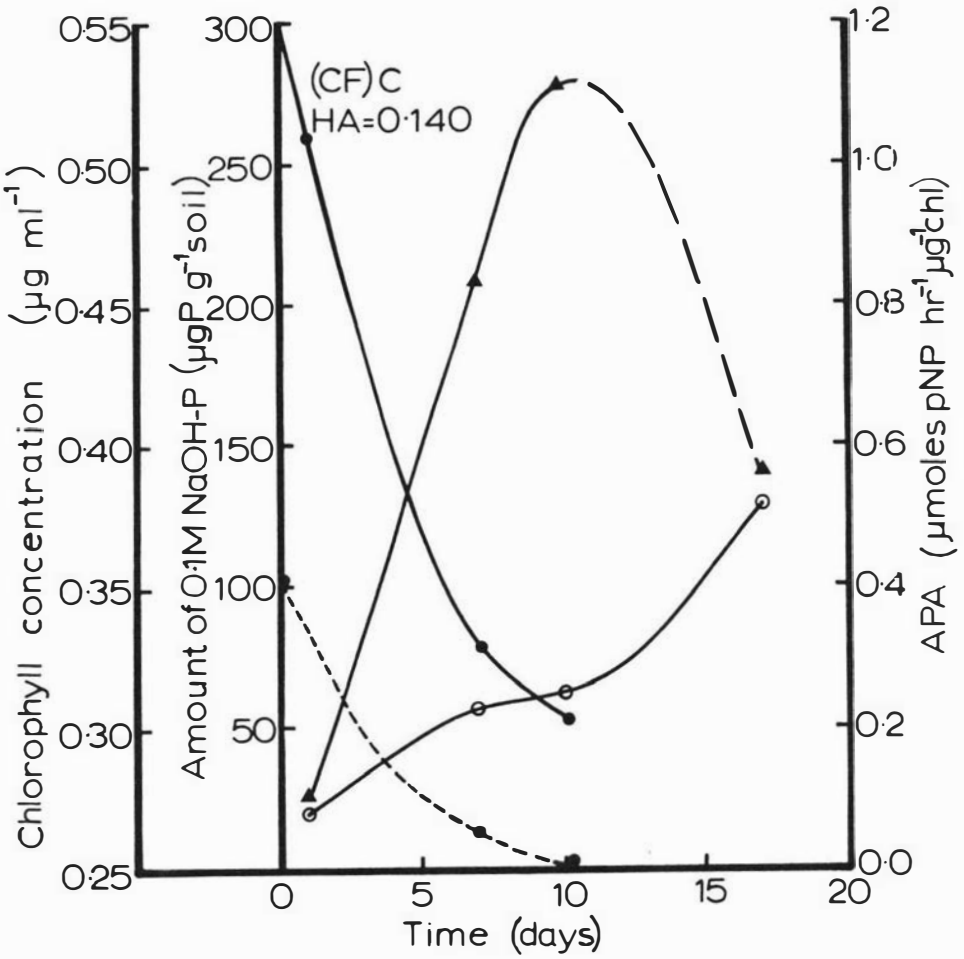


Fig. 6.11 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●—●), and alkaline phosphatase activity (APA) (—○—) during the growth of Anabaena in the non-shaken bioassay of Waimakariri soil (<30 µm). Dashed line (-▲-) represents chlorophyll pigment deterioration.

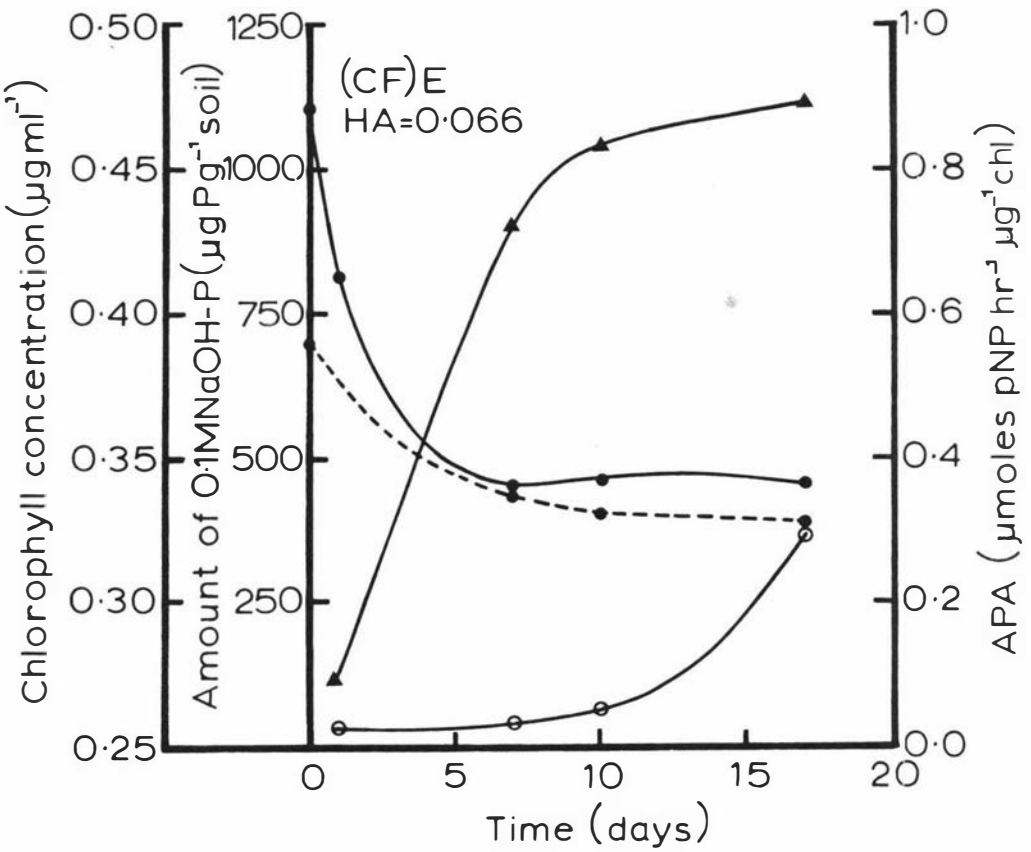


Fig. 6.12 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●···●) and -TP (●—●), and alkaline phosphatase activity (APA) (—⊖—) during the growth of Anabaena in the non-shaken bioassay of Egmont soil (<30 µm, 0.33g l^{-1}).

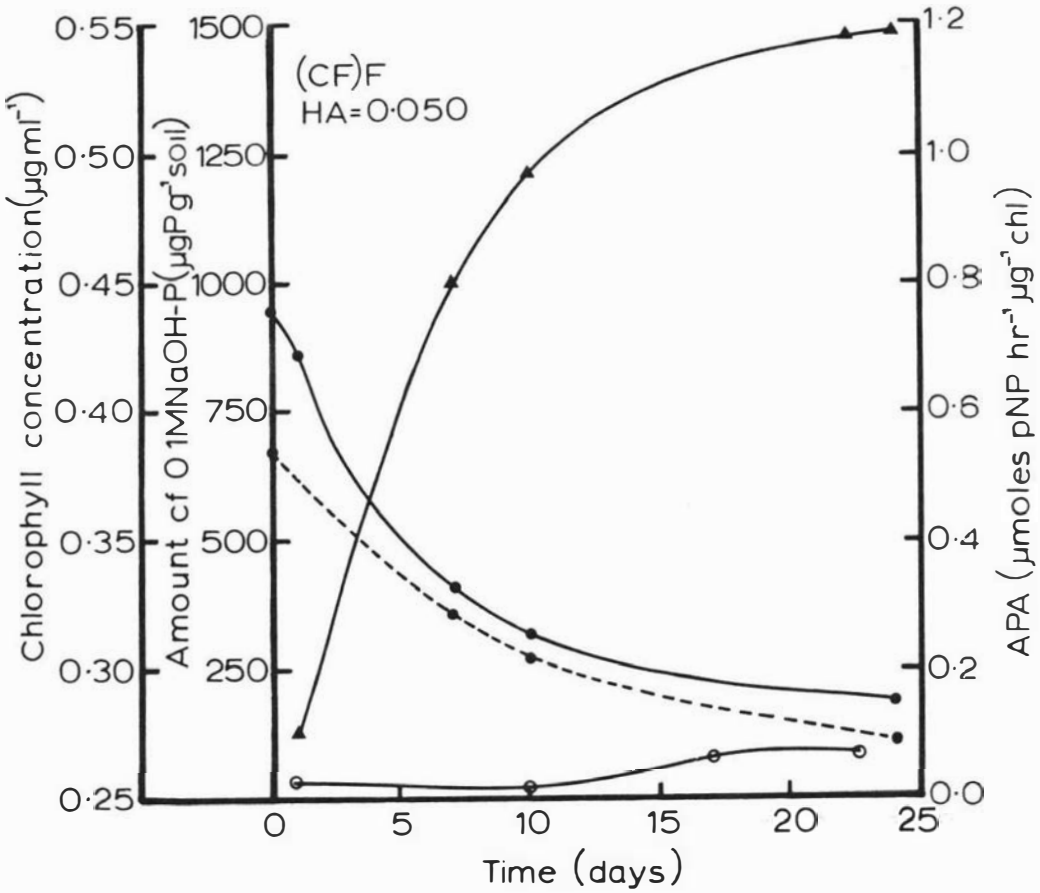


Fig. 6.13 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●——●), and alkaline phosphatase activity (APA) (—○—) during the growth of Anabaena in the non-shaken bioassay of Manawatu soil (<30 µm).

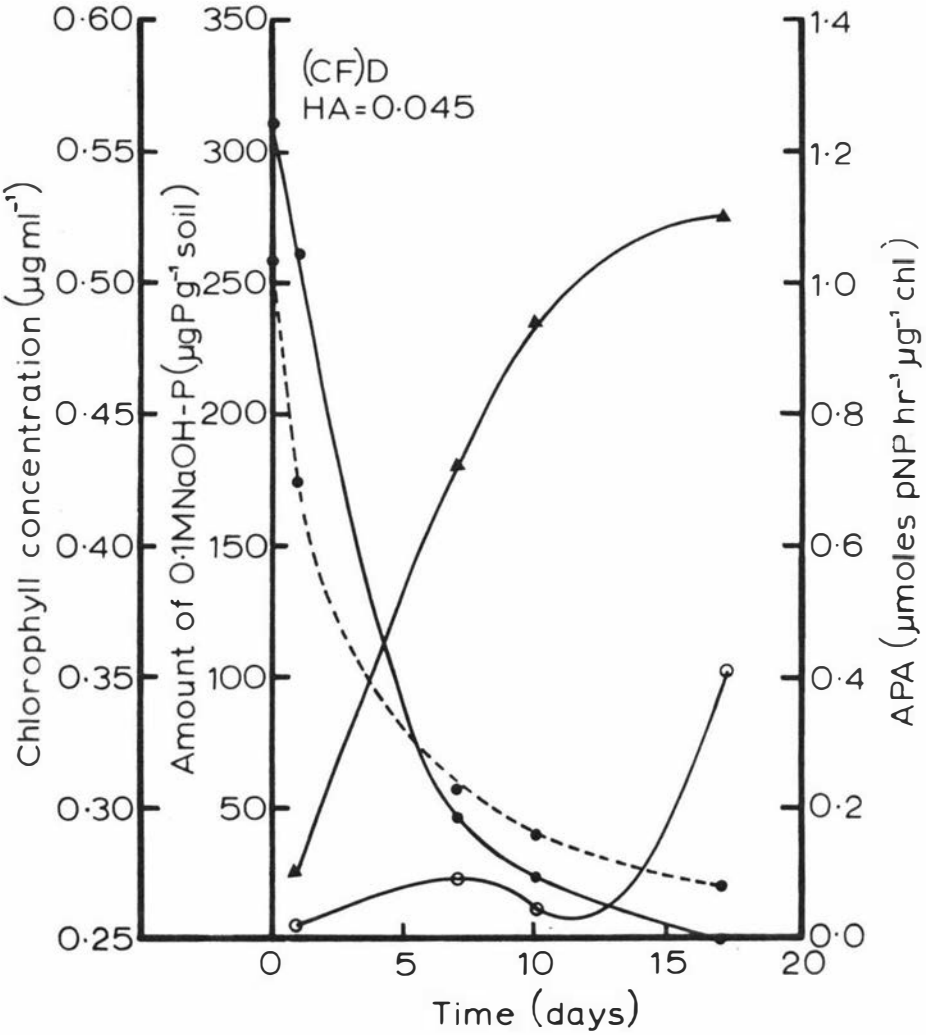


Fig. 6.14 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●—●), and alkaline phosphatase activity (APA) (—⊖—) during the growth of Anabaena in the non-shaken bioassay of Manawatu River sediment.

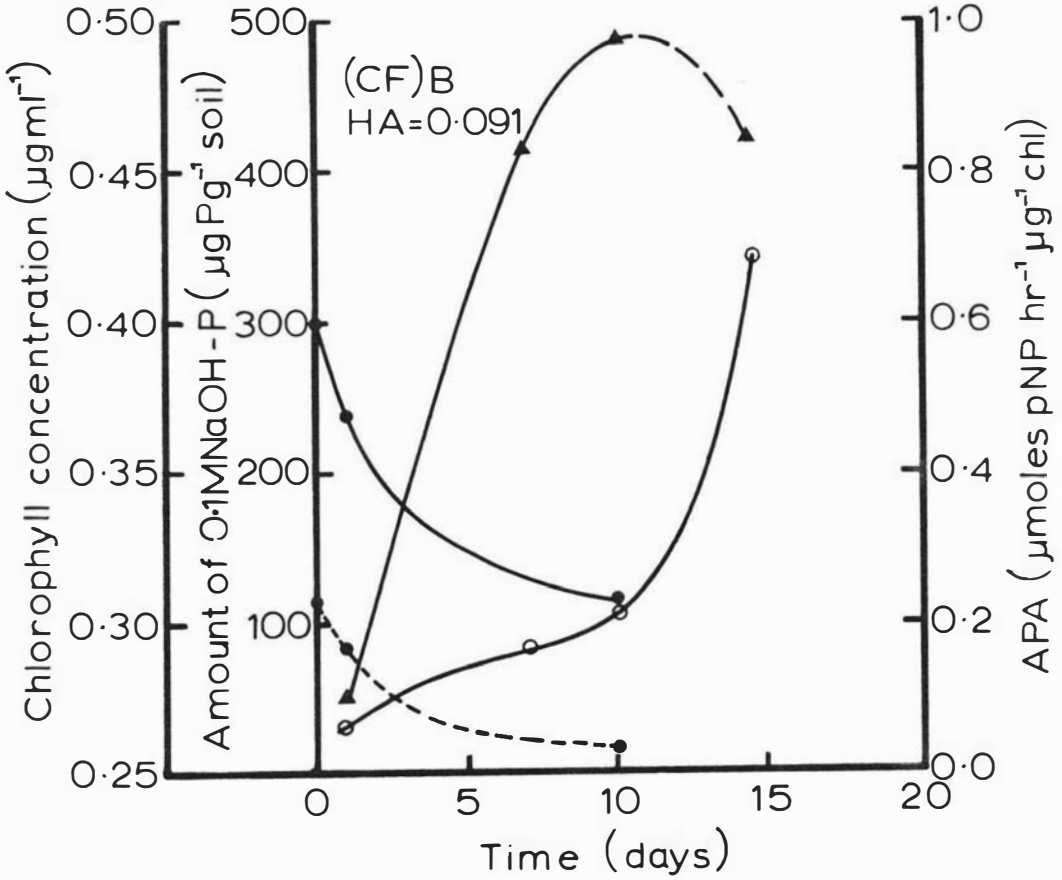


Fig. 6.15 Chlorophyll concentration (—▲—), amounts of 0.1M NaOH-IP (●- -●) and -TP (●—●), and alkaline phosphatase activity (—○—) during the growth of Anabaena in the bioassay of Atawhai soil (<30 µm). Dashed line (-▲-) represents chlorophyll pigment deterioration.

Nevertheless, similar trends in enzyme activity were observed. In all of the soil systems, alkaline phosphatase activity began to increase when the initally rapid rate of 0.1M NaOH-IP depletion from the soil decreased (Figs. 6.8 to 6.15). A further increase in enzyme activity was also observed at the end of the exponential growth-phase, when the rate of depletion of 0.1M NaOH-soil-TP decreased. The data in Table 5 (Appendix) were used to determine the algal P contribution to extractable-soil-algal P. The extractable P data for algae in Fe gel, control flasks, having similar alkaline phosphatase activity to algae in the soil systems, were plotted against the corresponding chlorophyll concentrations. In this way, estimates of extractable-algal P in the soil-algal systems could be obtained from the measured chlorophyll concentration. Correction curves of biomass (chl concentration) against extractable-algal P were then produced to cover algal growth on all soils (i.e., to cover algal growth under conditions of varying P availability).

With all soils, except Egmont, the 0.1M NaOH-IP fraction showed a slightly higher biological availability than the corresponding 0.1M NaOH-OP fraction (See Section 6.3.1.1). It was particularly evident that 0.1M NaOH-soil-OP exhibited lower biological availability in soils where the 0.1M NaOH extract contained a higher amount of humic material (as indicated by colour); namely Okaihau, Waiotu, Waimakariri, and Atawhai soils (Figs. 6.9, 6.10, 6.11, and 6.15). This suggests that humic-associated P in these soils shows low availability and that it may have constituted the major part of the non-available, 0.1M NaOH-soil-OP that remained after the bioassay of Waimakariri and Atawhai soil materials (Section 6.3.1.1). Vigorous algal growth, which resulted from the minimally P-starved inoculum in the bioassay of Waiotu soil material

(Section 6.3.1.2), however, appears to be conducive to the use of this more-resistant OP form as a P source.

The reduced availability of 0.1M NaOH-soil-OP in this study contrasted with the results from the shaken studies, in which 0.1M NaOH-soil-OP showed a slightly higher availability, in general, than 0.1M NaOH-soil-IP. This difference may have resulted from the different concentrations of alkaline phosphatase and perhaps other hydrolytic enzymes in the two systems, i.e., the greater algal growth in the shaken systems resulted in higher concentrations of alkaline phosphatase, which presumably would enhance the hydrolysis of soil OP.

Biologically-available P in all systems,except those containing <30-µm material from Egmont (Fig. 6.12) and Atawhai (Fig. 6.15) soils, accounted for 73 to 100% of the 0.1M NaOH-soil-TP fraction. The low availability of the 0.1M NaOH-IP fraction from the allophanic Egmont soil and the almost complete depletion of 0.1M NaOH-soil-OP are consistent with the findings reported in Section 6.3.1.1.

The 0.1M NaOH-TP fraction of the Atawhai soil showed low biological availability as obtained in the previous bioassay (Section 6.3.1.1). As reported previously (Section 6.3.1.1), OP was the major contributor to that part of the 0.1M NaOH-TP which was not utilized in the Atawhai soil. A number of factors probably contribute to produce a poor environment for soil-P utilization in the Atawhai soil; these are discussed in Sections 6.3.1.1 and 6.4.

In general, the data for the non-shaken bioassays confirm those for the shaken bioassays. It appears that even with minimally-disturbed sediment, the amount of biologically-available particulate P in all materials, except that from Egmont soil and to a lesser extent Atawhai

soil, can essentially be equated with that P extracted by 0.1M NaOH.

6.4 General Discussion

Although attempts have been made to quantitatively measure and characterize the amounts of biologically-available particulate phase IP in lake sediments (Golterman et al., 1969; Golterman, 1973b; Sagher, 1974), the few studies of soils and runoff materials have been limited to bioassays, which have only allowed estimates of biologically-available P to be made, but have not permitted characterization of the available forms (Healy and McColl, 1974; McColl, 1975; Cowen and Lee, 1976). The development, in the present study, of procedures for the combined bioassay-chemical fractionation of particulate P and for the simultaneous fractionation of algae, which were grown in similar P environments to those maintained by soil materials, has allowed a more accurate determination of the algal P contribution to soil-algal extracts and has made it possible to follow more closely the depletion of chemically-characterized soil P fractions.

Solution IP maintained by all materials was completely available for algal growth. In most cases, solution IP was assimilated during the first 1 to 3 days of a bioassay. The similar depletion of Solution IP by algal growth has been mentioned by several other workers (Rodhe, 1948; Mackereth, 1953; Hutchinson, 1957; McColl, 1972). Solution OP, where monitored (Tokomaru and Okaihau soils, Section 5) required a longer time for depletion by Anabaena. The availability of dissolved OP has been little studied, although Herbes et al. (1975) have shown that 50% of the soluble organic P in lake water was hydrolyzed by phytase. Also, alkaline phosphatase has been shown to release large amounts of DIP from organic

material in Lake Kinnerett, in northern Israel (Berman, 1969).

Over the wide range of soil materials bioassayed in this study, only the 0.1M NaOH-IP and -OP fractions were depleted by algal growth. The same form of particulate IP, referred to as non-occluded IP, has been shown to be depleted from lake sediments when biological production and demand for P in surface waters were high (Wildung et al., 1977). The work of Sagher (1974) and Golterman et al., (1969), as discussed in Section 5.3.3.4, has also shown that NaOH-extractable IP has a high biological availability in lake muds. Although they did not measure the depletion of particulate-phase P, Cowen and Lee (1976) also found that estimates of biologically-available P in the fine sediment of urban runoff were more closely correlated to those amounts of IP extracted by 0.1M NaOH and an anion-exchange resin than by an acid extraction.

In the present study, the particulate-phase OP, which is extracted by 0.1M NaOH, was found to be an important source of biologically-available P. During vigorous algal growth on the < 30-μm materials and on river and runoff sediments, OP supplied more P to Anabaena than particulate IP. It is possible that part of the 0.1M NaOH-soil-IP may have arisen from occluded soil IP. In general, in soils with higher amounts of Fe and Al components, the 0.1M NaOH-soil-IP fraction showed a lower biological availability (e.g., Waiotu, Egmont, and Atawhai, Fig. 6.10, 6.12, and 6.15, respectively). Occluded soil IP is probably unavailable for algal growth, as discussed in Section 6.3.1.1. The above mentioned factors could partly explain why 0.1M NaOH-soil-OP, which probably arises solely from surface-bound OP, generally shows a greater percentage availability than 0.1M NaOH-soil-IP under conditions of vigo rous algal growth.

Although, as discussed in Section 5.3.3.4, much evidence points to

the indirect availability of soil and sediment OP to higher plants and algae, the amounts and forms of OP which are potentially available have not previously been determined. Organic P is probably largely surface adsorbed because the organic moiety would hinder the diffusion of OP into amorphous soil components. In addition, the combined hydrophobic-hydrophyllic nature of the OP molecule may encourage surface coating rather than the development of concentrated areas of OP. At the soil surface, OP may be sorbed by the same Fe and Al oxides and hydrous oxides which sorb IP (Sommers et al., 1972; Anderson et al., 1974) or complexed with humic and fulvic acids (Section 9; Swift and Posner, 1972; Veinot and Thomas, 1972). A high proportion of this OP is probably susceptible to enzymatic hydrolysis, which could account for its high availability under conditions of vigorous algal growth.

The biological availability of soil OP is governed by the hydrolytic conditions imposed by the algae or other microorganisms. This is demonstrated by the fact that when a large inoculum was added, or when initial algal growth from either P stored in the inoculum or readily-available solution P, produced a large quantity of algae and higher concentrations of alkaline phosphatase, greater amounts of soil OP were depleted by algal growth. In the shaken studies, where algal growth was not vigorous, OP was not as readily hydrolyzed. This was particularly obvious with soils having a large amount of humic material associated with the 0.1M NaOH extract. As mentioned previously, this suggests that humic-associated P may be more resistant to enzymatic hydrolysis. This may be expected because it is known that IP and OP associated with humic material are involved in high molecular weight, organic-matter complexes (Thomas and Bowman, 1966; Lévesque, 1969; Swift and Posner, 1972).

Organic P held in these complexes may well have greater protection from hydrolytic enzyme action.

Most of the <30-µm materials showed a slightly lower availability of 0.1M NaOH-TP in the non-shaken, than in the shaken systems. The data from both studies are generally similar and show that between 70 and 100% of 0.1M NaOH-soil-TP is depleted by algal growth on the majority of soil materials. The exceptions to this are the allophanic Egmont soil, for reasons explained in Section 6.3.1.1, and to a lesser extent the Atawhai soil. The Atawhai soil probably contains a very small amount of weakly-adsorbed P, as discussed in Section 6.3.1.1. The 0.1M NaOH-TP fraction of the Atawhai soil shows a lower availability than that of the other basaltic soils; this could be explained by the following. The Atawhai soil has a lower NaOH-IP:P-sorbing components (Fe and Al) ratio than the other two basaltic soils, Waiotu and Okaihau (Williams and Walker, 1969a). This may suggest that surface IP is more strongly sorbed in the Atawhai soil and is less-readily desorbed at iso-pH (Ryden and Syers, 1977b) to provide P to initiate algal growth. A comparison of the fractionation data with that of Williams and Walker (1969a) indicates that by selecting <30- µm material from the Okaihau and Waiotu soils, the amount of CDB-P per unit weight of soil decreased but, with the Atawhai soil it increased. The CDB-P fraction is therefore associated with the finer material in the Atawhai soil and this would tend to lead to an increased extractability in NaOH. Thus the 0.1M NaOH-P fraction from the Atawhai soil may contain more IP derived from occluded soil P, than for the other soils. Thus, because occluded IP would be expected to have a low biological availability 0.1M NaOH-TP would overestimate biologically-available P in the Atawhai soil.

The CDB-IP and 1M HCl-IP fractions in the < 30-μm materials studied did not appear to be utilized by Anabaena, even under the optimum conditions that existed for algal growth and soil P depletion. The non-availability of these IP fractions contradicts the results obtained by Golterman et al. (1969) with Dutch lake muds (discussed in Section 5.3.3.4). Acid-extractable P, however, accounted for no more than between 1 and 26% of the extractable P in the < 30-μm material studied here whereas in the muds studied by Golterman et al. (1969) it accounted for 50% of the extractable P. In addition, the latter samples were supersaturated with respect to calcium phosphates. It is possible that this system would maintain a higher solution IP concentration than the acid-extractable P in the < 30-μm materials used here.

The chemical characterization of the soil IP that is available for the growth of Anabaena has provided a better understanding of the forms of soil IP which are biologically available. Firstly, P occluded within oxides and hydrous metal oxides (crystalline and amorphous forms) and present within calcium phosphates can largely be discounted as forms of biologically available soil IP, because 1M NaOH-P, CDB-P and 1M HCl-P fractions (Section 2.2.3.1) were little affected by algal growth. The large amount of 0.1M NaOH-IP which remained after algal growth on the allophanic Egmont soil (Section 6.3.1.1) indicates that IP occluded within amorphous aluminosilicates may also be expected to have a low biological availability. The major form of IP extracted by 0.1M NaOH, from non-allophanic soils, probably consists of P which is sorbed at the surface of Fe and Al components in soil (Williams et al.,1967; McLaughlin et al., 1977). The results obtained in this Section suggest that this form of soil IP is almost completely biologically available. Slight overestimates

of the amount of biologically-available IP present in a particulate material were believed to result from the extraction of small amounts of occluded IP by 0.1M NaOH from < 30- μm materials.

The forms of soil OP available for algal growth are less-easily identified. The nature of OP compounds in soil remains largely obscure and, in addition, 0.1M NaOH extracts a large amount of very complex organic material (Anderson and Malcolm, 1974). Apart from the functional groups, this material remains unidentified. Further investigations into the nature of biologically-available soil OP are described in Section 9.

The additional, more specific information gained by using a combined bioassay-chemical fractionation procedure can be illustrated for one material studied, Egmont black loam, for which it is possible to compare the results of this study with those obtained by McColl (1975). From the observed growth of Chlorella vulgaris, McColl estimated that 1125 μg P g^{-1} in the clay fraction of Egmont soil was available for algal growth. He was not able to characterize the form of soil P used, apart from establishing the fact that it was equivalent to 32% of 0.5M H_2SO_4-soil-IP, which gives no indication of the important role played by OP as a biologically-available P form in this soil (Fig. 6.12). By using 0.5M H_2SO_4 as the soil IP extractant it is also impossible to determine the origin of the available IP, because 0.5M H_2SO_4 is capable of removing varying amounts of all forms of soil IP, some of which have been shown to be biologically unavailable in the present study. Although slightly larger particle-size material was used in the present study, a similar amount (1185 μg g^{-1}) of Egmont soil P was available for algal growth. By using the combined bioassay-P fractionation procedure and by correcting for algal P contribution to soil-algal extracts it has been possible to

show that the available P consisted of 645µg of soil OP and 540 µg of soil IP, which probably originated solely from surface-sorbed soil P.

The data obtained in Section 6.3.1.3 clearly demonstrate that the ability of stream-bank material to supply P for algal growth is low compared to surface-runoff material. Surface-runoff contributed only 14% of the annual sediment load in the stream which drains the gently-sloping catchment, from which these materials were collected (Sharpley, 1977). The remainder of the sediment load (86%) originated from a small amount (16%) of sediment carried into the stream by subsurface flow (mostly from tile drainage) and from large inputs (70%) of stream bank and resuspended stream-bed material. If it is assumed that the biological availability of particulate-phase P in stream-bank material is similar to that in subsurface flow and stream-bed material, then by directly relating the sediment loads to the amounts of P in each material it can be seen that although only supplying 14% of the stream-sediment load, surface-runoff material provides 33% of the biologically-available particulate phase P carried by the stream. Consequently, while it is impossible to control sediment inputs from stream bank and resuspended stream bed material, the control of topsoil erosion, even in gently-sloping catchments, may considerably reduce the amounts of biologically-available P entering streams.

Before any schemes for the control of P inputs into waters can be developed it would be desirable to identify those sources of particulate P which have a high biological availability. It has been shown that the extraction of a water sample containing fine soil materials with 0.1M NaOH provides a rapid and simple procedure for estimating the amount of P in the sample that is potentially available for algal growth. Using

this procedure it should be possible to determine the biological availability of sediment loads entering a lake. To achieve this, the following procedure is proposed. To a 35-ml aliquot of unfiltered stream water add 5 ml of 0.8M (NaOH + NaCl). After a suitable extraction time (long periods of extraction are not required, Section 5.3.2.1), the IP determined following ammonium persulphate digestion would provide an estimate of the P which has a very high potential biological availability. If allophanic soil materials were present, care would be required in the interpretation of the results.

SECTION 7

COMPARISON OF ALTERNATIVE METHODS FOR THE DETERMINATION OF BIOLOGICALLY-AVAILABLE PARTICULATE P

7.1 Introduction

In the previous Section it was established that the amount of P extracted from potential surface runoff materials and stream-sediment source materials by 0.1M NaOH was essentially the same as that which could be used by Anabaena under the bioassay conditions employed. The purpose of this Section is to compare this fraction of soil P with other estimates of soil P which have been used to assess biological availability. O'Connor and Syers (1975) investigated the forms of particulate P extracted by digestion with ammonium persulphate and found that the P in the soil residue after digestion was largely in the occluded form. The persulphate digestion, therefore, extracts mainly organic P and non-occluded IP forms and apatite if present. Thus, it may be expected that the P recovered by persulphate digestion has a high biological availability. If so, it would provide a simple and rapid method for determining the amounts of potentially biologically-available P in a water sample containing particulates. A simple comparison between the amounts of P extracted by persulphate digestion and 0.1M NaOH from four representative soils was carried out.

Isotope dilution studies have widely been used for the estimation and study of plant-available IP in soils (Talibudeen, 1957; Amer, 1962; Jose and Krishamoorthy, 1972; Ryden and Syers, 1977b). The amount of "labile" IP is usually defined as the sum of solution IP and isotopically-exchangeable or surface IP that exists in equilibrium with the soil solution.

It may be expected that this form of P is readily available for algal growth (Li et al., 1972), whether in a lake or a runoff environment. The second part of this Section is concerned with a comparison of the amounts of isotopically-exchangeable P and 0.1M NaOH-IP in soils in an attempt to characterize further the nature of 0.1M NaOH-soil-IP. Also, the extent to which values of exchangeable soil P reflect the amount of soil P available for algal growth during the bioassay procedure, previously employed, is evaluated. Previous work by Dunbar and Baker (1965) and Tandon and Kurtz (1968) has shown that soil IP extracted in a single NaOH extraction shows a high degree of exchangeability. Li et al. (1973) also demonstrated that the amounts of exchangeable P in lake sediment were closely related to the amounts of non-occluded IP. Thus, it may be expected that the amount of 0.1M NaOH-TP would overestimate the amount of exchangeable soil P, due to the extraction, by NaOH, of forms of P with a low exchangeability (e.g., organic P and possibly discrete forms of Fe and Al phosphates). It is likely, however, that exchangeable soil P values derived from long-term incubation studies and values for native sorbed P, calculated by the method of Ryden et al. (1977a), may approach the values of 0.1M NaOH-soil-IP. Such values may provide an alternative method for estimating the amount of soil IP available for algal growth.

7.2 Materials and Methods

The < 30-μm material from the topsoils of Manawatu, Okaihau, Tokomaru, and Egmont, was used in the study comparing the amounts of P extracted by persulphate digestion and 0.1M NaOH. In the comparison between isotopically-exchangeable P and 0.1M NaOH-IP, the < 30-μm material from the above mentioned four soils, Waiotu, Atawhai, and Wairiakariri, soils and earthworm casts was used.

7.2.1 Ammonium persulphate digestion

The soil materials were suspended in distilled water (1g/200ml) using a magnetic stirrer. Aliquots (25ml) were pipetted into three 50-ml Erlenmeyer flasks. Each sample was acidified with 0.5ml of concentrated H_2SO_4, 0.3g of ammonium persulphate was added, the flask sealed with aluminium foil, and autoclaved at 120°C for 45min. The cooled digest was washed with distilled water into a 40-ml polypropylene, screw-cap centrifuge tube and the soil separated by centrifugation. The supernatant solution was decanted into a 50-ml volumetric flask, neutralized with 5N NaOH (p-nitrophenol was used as the indicator), and made up to volume with distilled water. The P concentration was determined on a suitable aliquot.

A similar persulphate digestion was also performed on the residue that remained after the soil material had been extracted with 0.1M NaOH. The 0.1M NaOH extraction is described in Section 3. Both IP and TP concentrations were measured in the combined extract and 1M NaCl washings.

7.2.2 Isotopic exchangeability

The amount of soil P which was exchangeable in 30 min was determined using soil suspended (0.1g/40ml) in 0.1M NaCl containing mercuric chloride ($HgCl_2$, 50mg l^{-1}), that had been preshaken for 40hr. Carrier-free ^{32}P was added in a 1-ml aliquot to triplicate samples of the suspended materials. After 30 min contact between added ^{32}P and the soils, the tubes were centrifuged and solution IP concentration was determined. Solution ^{32}P concentration was determined by adding 10ml of Triton-toluene scintillation cocktail[1] to a 0.2-ml aliquot of the aqueous extract in a

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[1] The Triton cocktail was prepared by dissolving 4g of PPO and 0.1g of MP_2POPOP in 667ml of toluene and making up to 1 litre with Triton X-100.

vial and counting in a Beckman LS 350 Liquid Scintillation Spectrometer. Blank aqueous extracts of each soil were used for quench correction. Exchangeable P was calculated from isotope dilution theory. Soil P was extracted with 0.1M NaOH. Extractable-IP was determined as described in Section 3 and extractable ^{32}P was determined as above. Blank 0.1M NaOH extracts of each soil were used for quench correction.

An estimate of native sorbed IP was determined graphically from values of exchangeable P for ^{32}P-soil contact times of 3, 4, 5, and 6 days (Ryden and Syers, 1977a). Each system was shaken for the same period of time because ^{32}P was added 6, 5, 4, and 3 days before the measurement of solution ^{32}P and IP and 0.1M NaOH-soil-^{32}P and -IP. Solution ^{32}P measured at each time interval was plotted against the reciprocal of time and the resulting linear relationship was extrapolated to $^{1}/_{t} = 0$ (infinite time) to give an estimate of equilibrium ^{32}P concentration. Using isotope dilution theory, native exchangeable P at equilibrium was calculated. The value obtained was assumed to be an estimate of native sorbed IP (Ryden and Syers, 1977a).

7.3 Results and Discussion

7.3.1 Particulate P extracted by persulphate digestion

With the exception of the Egmont soil, from which 0.1M NaOH would be expected to extract P occluded within allophane, persulphate digestion of the materials extracted approximately twice the amount of TP removed by the 0.1M NaOH extraction (Table 7.1). Following a 0.1M NaOH extraction (A), persulphate digestion (B) of the residue extracted 110, 65, 99, and 24% of 0.1M NaOH-TP from the Manawatu, Okaihau, Tokomaru, and Egmont soils, respectively. The combined amount of TP removed by both extractants

Table 7.1 Amounts of P extracted by persulphate digestion, 0.1M NaOH, and reagents of the fractionation scheme (total extractable P) from <30-μm material of four soils

Material	Amount of P extracted (μgP g^{-1}) by					
	Persulphate digestion	0.1M NaOH (A)		Persulphate digestion of residue from A (B)	Total P extracted (A + B)	Total extractable P (A + 1M NaOH + CDB + HCl)
		IP	TP			
Manawatu	1851	790	955	1060	2015	1945
Tokomaru	415	84	242	240	482	422
Okaihau	403	108	255	167	422	400
Egmont	4272	2190	3282	789	4071	4230

(A + B) was similar to the amount of soil TP recovered by a single persulphate digestion for all soils. This suggests that the TP removed by persulphate digestion includes the same TP extracted by 0.1M NaOH. It is reasonable to expect that the hydrous oxides of Fe and Al, which are involved in the sorption of P, would be attacked by the acid in the persulphate digestion reagent. The strong oxidizing conditions of the digestion would also remove soil OP, including 0.1M NaOH-soil-OP (Menzel and Corwin, 1965).

When the amounts of P extracted subsequent to the 0.1M NaOH extraction in the fractionation procedure (Table 6.2) were added to the amounts extracted by 0.1M NaOH (Table 7.1), the total obtained was very similar to the amount of P extracted by persulphate digestion. The results suggest that persulphate digestion is capable of removing all the forms of soil P that are extracted by the fractionation procedure (Table 6.1).

7.3.2 Exchangeable particulate phase P

In short-term incubations involving $HgCl_2$ addition, ^{32}P would be expected to exchange only with soil IP, and not with soil OP. The amounts of soil IP previously shown to be potentially available for algal growth (i.e., 0.1M NaOH-extractable-IP) were therefore compared with the amounts of isotopically exchangeable P.

The amount of soil IP which was exchanged in 30min was only 4 - 10% of the amount of IP extracted from the soil by 0.1M NaOH (Table 7.2). Three soils (Okaihau, Waiotu, and Atawhai), however, showed a greater proportion of exchangeable P, but this possibly resulted from a procedural error which is discussed later in this Section. Within experimental error, after a 30-min exchange period there was total recovery of ^{32}P from the

Table 7.2 Amounts of 0.1M NaOH-extractable IP and of exchangeable P as a function time allowed for exchange in <30- μm material of several soils

Material	0.1M NaOH-extractable IP ($\mu g\ g^{-1}$)	Exchangeable P ($\mu g\ g^{-1}$) at			
		30 min	3 days	6 days	Equilibrium[1]
Tokomaru	90	9	54	61	70
Manawatu	746	56	226	307	418
Okaihau	110	32	298	318	337
Egmont	2340	107	589	750	1050
Waiotu	150	25	130	273	3930
Atawhai	140	19	87	141	292
Waimakariri	156	11	52	67	87
Earthworm casts	422	36	162	242	410

[1] Estimate of native sorbed IP.

soil by 0.1M NaOH (Table 7.3). This indicated that the soil IP characterized by 30min exchange is a fraction of 0.1M NaOH-IP and that in order for such rapid exchange to take place, this IP must be very weakly held at the soil surface (Ryden and Syers, 1977b).

The Okaihau, Waiotu, and Atawhai soils maintained lower solution IP concentrations (Table 7.3) than the Tokomaru or Wairiakariri soils but conversely, had higher levels of exchangeable P (Table 7.2). These three soils have a higher P sorption capacity and therefore added ^{32}P may be sorbed rather than exchanged. Also, as shown later, the determination of solution IP may well overestimate the true concentration of solution IP and the amount of solution IP capable of undergoing exchange. This would lead to an incorrect, calculated value for exchangeable soil IP.

When the data for 0.1M NaOH-IP were plotted (Fig. 7.1) against those for 30-min exchangeable P (data reflecting surface exchangeable P and no tracer occlusion), a close relationship was only obtained when the allophanic Egmont soil and the three basaltic soils were excluded; that is when the mineral composition of the soils was similar. This result supports the thinking that 0.1M NaOH-IP is derived from surface-sorbed IP, because for soils of similar mineral composition a direct relationship would be expected between the amount of total surface sorbed IP and the amount of weakly-sorbed surface IP. The data for the basaltic soils, which contain varying amounts of P-sorbing components (Williams and Walker, 1969a), do not show this relationship. As mentioned earlier, it is believed that the results for the basaltic soils were subject to error.

When longer periods of time were allowed for exchange (3 days and 6 days, Table 7.2) the values obtained for exchangeable P were still considerably lower than the amounts of 0.1M NaOH-soil-IP, except for the

Table 7.3 Comparison between amount of measured solution IP and solution IP calculated by using the ratio of solution ^{32}P:soil ^{32}P at equilibrium, and the effect of exchange time on the recovery of soil ^{32}P by 0.1M NaOH

Material	Solution ^{32}P:soil ^{32}P at equilibrium	Solution IP ($\mu gP\ g^{-1}$ soil)		Percentage of soil ^{32}P extracted by 0.1M NaOH after		
		Measured	Calculated	30 min	3 days	6 days
Tokomaru	0.163	11.4	18.2	100	95	93
Manawatu	0.289	121	215	96	96	93
Okaihau	0.002	0.62	0.198	100	94	92
Egmont	0.006	6.0	13.3	100	97	95
Waiotu	0.0002	0.95	0.03	100	93	89
Atawhai	0.013	2.8	1.82	100	86	83
Waimakariri	0.203	17.8	31.6	97	91	91
Earthworm casts	0.075	31	31.6	100	96	92

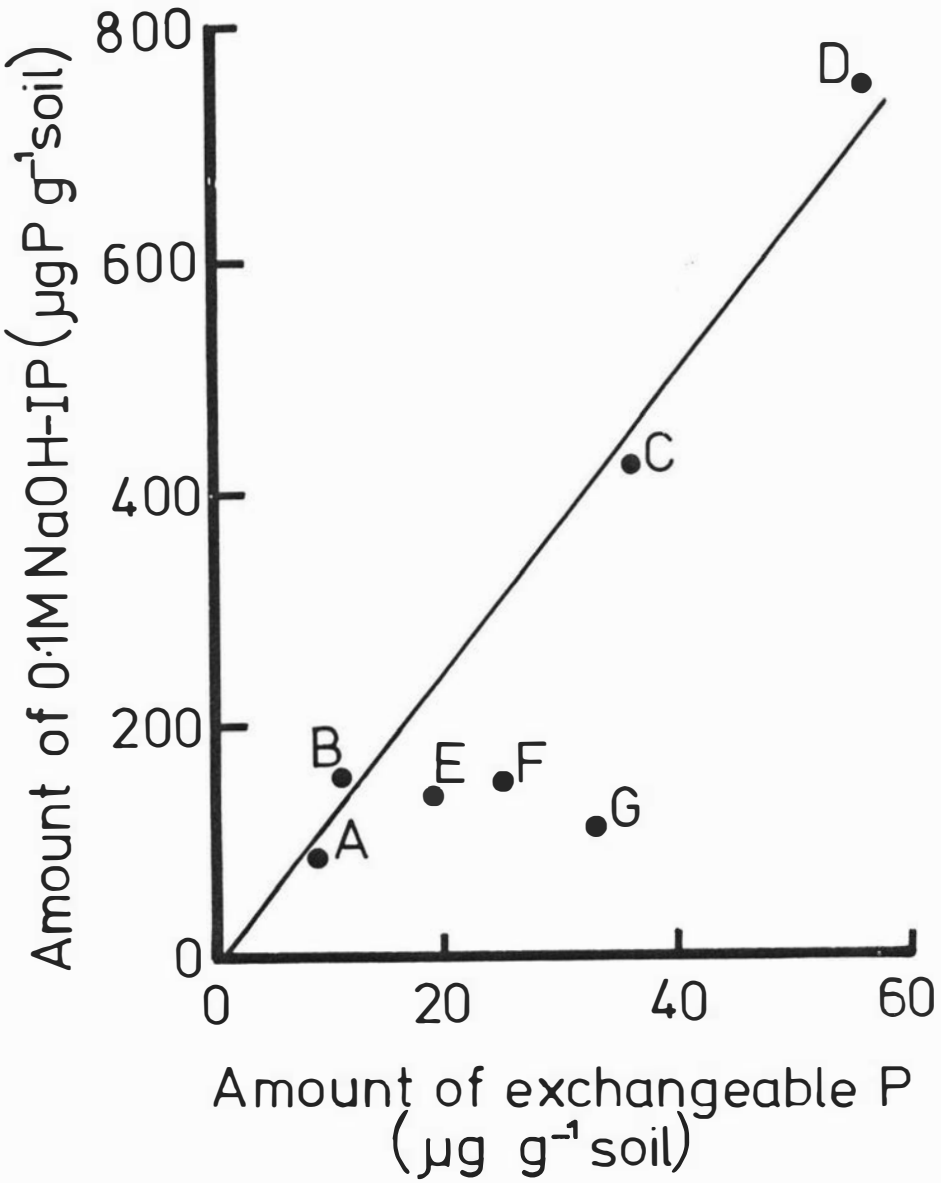

Fig. 7.1 Relationship between amounts of 0.1M NaOH-IP and exchangeable P (30 min) for the <30-µm material from Tokomaru (A), Waimakariri (B), Manawatu (D), Atawhai (E), Waiotu (F) and Okaihau (G) soils, and from earthworm casts (C). Line drawn only through points A, B, C, and D.

basaltic soils. At these longer periods of incubation it was not possible to recover, by 0.1M NaOH extraction, the total amount of ^{32}P sorbed by the soil and the percentage recovery decreased with increased time of contact (Table 7.3). This phenomenon has also been observed by Ryden et al. (1977b) who only obtained complete recovery of ^{32}P sorbed by soils, Fe gel, and natural goethite, when CDB and 1M HCl extractions were made subsequent to NaOH. This implied that ^{32}P had diffused into short-range order hydrous ferric oxides, becoming absorbed or occluded (McLaughlin et al., 1977), from where it could not be extracted by 0.1M NaOH. It has previously been shown (Sections 5 and 6) that this form of occluded P is biologically unavailable. The fact that absorbed or occluded ^{32}P is not extracted by 0.1M NaOH confirms the theory that very little native soil IP occluded within hydrous ferric oxides is actually extracted by 0.1M NaOH. Another interesting feature of the ^{32}P recovery data (Table 7.3) is that for the Egmont soil, where it was suggested that 0.1M NaOH dissolved allophane and therefore extracted IP occluded within it (Section 6; Williams and Walker, 1969b), the percentage of ^{32}P recovered was higher than for the other non-allophanic soils. It is also noticeable that the Atawhai soil which had the highest value for CDB-P (Table 6.2) also gave the lowest value for ^{32}P recovered in 0.1M NaOH, suggesting that ^{32}P absorption may be related to the amount of hydrous ferric oxides present in a soil.

The exchangeable P values calculated for the Okaihau, Waiotu, and Atawhai soils are subject to error, as shown by the very high values calculated for native sorbed IP (Table 7.2). All three soils gave a low recovery of ^{32}P after 6 days (Table 7.3), which suggests that ^{32}P was being absorbed as well as exchanged. This would result in the over-

estimation of exchangeable soil P. The amount of absorbed tracer is not sufficient, however, to account for the large overestimate of exchangeable P. When extrapolated to infinite time, the value obtained for the ratio of solution ^{32}P:soil ^{32}P should correspond to the equilibrium ratio of solution $I^{31}P$:total exchangeable soil $I^{31}P$. At this point it would be expected that all surface sorbed IP would be exchangeable. Consequently, the value of 0.1M NaOH-IP could be taken as an estimate of total exchangeable $I^{31}P$ at equilibrium. Ryden and Syers have used both exchangeable P at equilibrium (1977a) and 0.1M NaOH-IP (1977b) to estimate the amount of native sorbed IP present in a soil. Using 0.1M NaOH-IP as an estimate of exchangeable P at equilibrium and the distribution ratio of ^{32}P at infinite time, it was possible to calculate a theoretical value for solution IP sustained by the soils. The data indicate that, for the majority of soils, 0.1M NaOH-IP overestimates exchangeable P at equilibrium, as shown in Table 7.2. Thus, the calculated solution IP values also exceed the measured solution IP in all cases, except for the Okaihau, Waiotu, and Atawhai soils, for which the calculated solution IP is considerably lower than the measured value. This may suggest that the measurement of solution IP in these soils overestimates the actual amount of IP in solution which can freely exchange with the surface. Overestimation of solution IP may occur with all soils but it would constitute a larger error with these three soils which maintain particularly low solution P concentrations. The error could result from the acid hydrolysis of soluble OP in the Murphy and Riley reagent (Murphy and Riley, 1962) or from P associated with colloidal Fe material that had passed through the 0.45μm membrane filter. It may be significant that all three soils contained higher proportion of NaOH-extractable OP and of Fe components than the other soils studied (Table 6.2). Rigler (1968) has

also suggested that dissolved OP compounds are hydrolyzed during the determination of orthophosphate in lake water samples by the molybdenum blue method and that this leads to overestimation of the orthophosphate concentration.

Other than for the three basaltic soils, the calculated values for native sorbed IP appear to be reasonable, (Table 7.2), presumably because with the higher solution IP concentrations maintained by these soils, the percentage error in solution IP measurement would be lower. The values for 0.1M NaOH-IP are higher than the calculated native sorbed IP values (Table 7.2). Assuming the results are valid, this suggests that a certain amount of the 0.1M NaOH IP fraction, while being biologically available, is non-exchangeable with ^{32}P. Because it was suggested earlier in the Section and by Williams et al. (1967) that, 0.1M NaOH extracts very little P occluded within hydrous ferric oxides, this non-exchangeable IP may be in the form of discrete P compounds with low P exchangeability, which are solubilized by NaOH, or organic P which is hydrolyzed during the extraction (see Section 9). It is further possible that some of the soil IP is prevented from exchange by being complexed with iron in soil organic components, which are removed by the NaOH extraction. Tokomaru soil and earthworm casts derived from the Tokomaru soil showed a much closer relationship between calculated native sorbed-IP and 0.1M NaOH-IP, suggesting that less non-exchangeable, 0.1M NaOH-IP is present in the Tokomaru soil. It may be significant that the Tokomaru soil contains low proportions of NaOH-extractable OP and short-range order hydrous ferric oxides (Table 6.2).

With the errors involved in the calculation of exchangeable P, the values obtained for native sorbed-IP can not be considered to be anything more than approximate estimates of surface-bound-IP.

7.4 General Discussion

At the low soil:solution ratio used in this present study, the amount of P removed from < 30-μm material by persulphate digestion approached the total amount of particulate P present. O'Connor and Syers (1975) have shown that persulphate digestion extracts more than 80% of the total P present in < 63-μm material when low soil:solution ratios are employed, and that the percentage of P extracted increases as the particle size decreases. The results presented in Table 7.1 suggested that persulphate digestion removed all the forms of chemically-fractionated soil P (Table 6.2), even occluded P extracted by the CDB reagent. Although O'Connor and Syers (1975) found that CDB-P showed low extractability in persulphate digests, very little CDB-extractable P remained in the residues from small quantities of their finer soil fractions. Because persulphate digestion extracts forms of P from <30-μm material which have a limited or no biological availability, namely, those forms of P extracted by 1M NaOH, CDB, and 1M HCl subsequent to a 0.1M NaOH extraction, then it would not be a suitable extraction procedure for the estimation of biologically-available P in a runoff or stream sample containing particulates. If the digestion procedure involves the same ratio of persulphate to suspension as used in this study, however, then the amount of P extracted from an unfiltered stream or lake sample containing a low concentration of fine particulates (< 30μm) may be considered as a reasonably accurate estimate of the total amount of P in the sample that could be extracted by the chemical fractionation procedure used herin.

Several authors have used short-term incubation studies to study exchangeable P, plant-available P, and labile pools of P in soils (Talibudeen, 1957; Ivanov and Stanev, 1972; Jose and Krishnamoorthy, 1972;

Ryden and Syers, 1977b) and exchangeable P in lake sediments (Li et al., 1973). Longer incubation times are considered to lead to side reactions such as crystal growth (Amer, 1962) and occlusion of the radioactive P (Ryden et al., 1977b). From the experimental data obtained in this study it is evident that whereas, 30-min exchangeable IP would be readily available for algal growth, this amount of P would considerably underestimate that available during a bioassay. Although short-term exchangeable P values do not indicate overall soil P availability to algae, the 30-min values if added to the solution P concentration values could provide a useful estimate of P available for immediate uptake by an algal population. Ryden and Syers (1977b) have shown that the amounts of 30-min exchangeable soil P and water-extractable P are similar. It is shown in Section 8 that this readily available form of P is very important both in the development of algal bloom and in the eventual amount of particulate P utilized subsequently by algae. Because only 4 - 10% of the 0.1M NaOH-soil-IP (i.e., that soil IP which is potentially-biologically available) is in the form of labile soil IP characterized by exchange with ^{32}P during 30min, mechanisms other than simple desorption must be responsible for the depletion of soil IP during a bioassay (Sections 5 and 6).

The results in Table 7.2 suggest that the values obtained for exchangeable P are subject to considerable error with poorly-fertilized soils, namely Okaihau, Waiotu, and Atawhai soils. As discussed previously, the error is believed to arise from the overestimation of the low solution-IP concentrations. The accurate determination of solution IP is essential for the determination of exchangeable P. Talibudeen (1957) employed very low concentrations of citrate ions (0.001 M) to raise the

solution P concentration of low P status soils, without affecting the measurement of labile P (exchangeable P). For soils with high P sorption capacities, such as Okaihau, Waiotu, and Atawhai, where solution P is very low and the ratio of solution P to soil P is very small, a small error in the measurement of solution P would produce a large error in the calculation of exchangeable P. McConaghy et al. (1966) have also obtained very high estimates of labile P for some basaltic soils with high P sorption capacities. Amer et al. (1969) observed that this error was accentuated when ^{32}P tracer was used in the absence of a ^{31}P carrier solution. In the latter study, the labile P present in soils of high P sorption capacity had a higher specific activity than the P remaining in solution at the end of the isotope exchange reaction. This led to an error in the calculation of exchangeable P because for accurate determinations of total exchangeable P, the activities for solid and solution phase exchangeable P must be the same at equilibrium. Amer et al. (1969) explained this ^{32}P enrichment of surface P by the recrystallization of Fe phosphates and suggested that this accounted for the error in calculated exchangeable P values. The time of tracer-soil contact, however, was only 30min, during which time it is unlikely that sufficient recrystallization, if it in fact occurs, or absorption could have taken place to account for such large errors. It would seem more likely that the lower specific activity of solution P, when compared with surface P, was due to over-estimation of the solution ^{31}P by the Murphy and Riley method. The fact that the error was significantly decreased by the addition of carrier ^{31}P with the tracer supports this theory. Carrier ^{31}P would increase the solution P concentration and would therefore reduce the percentage error involved in the determination of solution P. On the other hand, the

addition of carrier ^{31}P would not decrease the rate of recrystallization of any P at the surface. Long-term incubation of soils of high P sorption capacities would give even greater problems because the adsorption of ^{32}P would give rise to a decreasing solution P concentration, which together with the increased specific activity of soil P, would introduce much greater errors into the calculation of soil exchangeable P.

Where solution IP concentrations are such that they allow reasonably accurate exchangeable soil P values to be calculated, the values obtained probably provide a reasonable estimate of the soil IP which is highly available for algal growth. These values, however, considerably underestimate the amounts of soil IP that can become available for algal growth in the longer term and do not provide any estimate of soil organic P availability. Long-term incubations are also complicated by absorption of ^{32}P, yielding a form of soil P which has been shown to have limited biological availability.

SECTION 8

FACTORS INFLUENCING THE AVAILABILITY OF PARTICULATE PHASE PHOSPHORUS

8.1 Introduction

It is apparent from the results obtained in Sections 5 and 6, and from other studies (Golterman et al., 1969; Sagher, 1974; McColl, 1975; Cowen and Lee, 1976), that the amount of P which becomes available for algal growth can constitute a considerable proportion of the total P present in a particulate P source. Consequently, the release of this P to algae cannot reasonably be explained by the simple desorption of P from the soil or lake mud. The finding, in Section 7, that labile IP constituted only 4 - 10% of the soil IP available to algae during the bioassay procedure, also suggests that simple desorption plays only a minor role in determining the availability of soil P to algae.

The aim of this Section is to evaluate some of the factors and mechanisms which are potentially important in the utilization of particulate P by algae. It is believed that only by obtaining a better understanding of such interactions can control procedures, which will limit the ability of particulates to supply P for algal growth, be developed.

The initial part of this aspect of the study is concerned with determining whether soluble, extracellular algal products play any role in the desorption or hydrolysis of particulate-phase P. Chelating agents (Fogg and Westlake, 1955; Murphy and Lean, 1976) and hydrolytic enzymes (Berman, 1969, 1970; Fogg, 1973; Heath and Cooke, 1975) produced by algae could be important in the utilization of particulate-phase P by algae. Initial results suggested that algal cell-particulate phase P contact was an important factor in the utilization of particulate phase-P. This

aspect, along with the effect of initial solution P concentration and the effect of sterilizing the particulate material on soil P availability to algae, were studied.

8.2 Materials and Methods

8.2.1 Desorption studies

The < 30-μm particle size fractions of Manawatu silt loam and Okaihau gravelly clay were used in this study, being examples of the extremes of P status (Manawatu silt loam = high; Okaihau = low). The soils were suspended at a soil:solution ratio of 1:200 in 250-ml, screw-capped, polypropylene centrifuge tubes. Four experimental systems were established in duplicate for each soil type:

(1) Soil suspended in ASM-1, P-, N- media
(2) Soil + Fe gel (15mg, pH 7, enclosed in dialysis tubing) suspended in ASM-1, P-, N- media
(3) Soil + Fe gel (15mg, pH 7, enclosed in dialysis tubing) suspended in the filtrate from a P-starved algal culture
(4) Soil suspended in the filtrate from a P-starved algal culture

The filtrate (< 0.45 μm Millipore) was from a 2-week old algal culture that had reached the stationary phase of growth because of P deficiency. The soluble alkaline phosphatase activity of the filtrate was 0.08 μ moles p nitrophenol $hr^{-1}ml^{-1}$. All systems were originally adjusted to pH 7. Solution IP and 0.1M NaOH-soil-IP and -TP were determined on a suitable aliquot immediately after the establishment of

the systems. The tubes were shaken on an end-over-end shaker for 7 days at 24°C in the dark. After shaking, solution IP, 0.1M NaOH-soil-IP and -TP, and pH were determined.

To further investigate the effect of soluble, algal extracellular products on the desorption of soil IP, a sequential extraction procedure, using ASM-1, P-, N-, media and the filtrate from a P-starved algal culture, was employed to desorb P from Manawatu, Tokomaru, and Okaihau soil materials. Quadruplicate samples of each soil (0.04g of <30-µm material) were shaken for three consecutive 17-hr periods in 20-ml aliquots of the extractants. The filtrate from P-starved algal cultures was freshly filtered from a culture in stationary phase before each extraction. The average soluble alkaline phosphatase activity of the filtrate was 0.09 µ moles p nitrophenol hr^{-1} ml^{-1}. After each extraction, the supernatant solution was removed by centrifugation and filtration, and solution IP concentration determined.

8.2.2 Algal-soil contact study

The design of the diffusion cells which were used to separate soil and algae yet allow the free movement of soluble material and solution between the two, is illustrated in Fig. 8.1. Initially the diffusion cells were clamped together using galvanized steel bolts. In preliminary experimental studies the bolts were found to adversely affect algal growth, causing loss of pigmentation and death after only a few days of incubation. The bolts quickly became corroded, developing a grey powder-like coating within a week. The bolts were replaced by PVC, peristaltic-pump tubing, the elasticity of which enabled the cell components to be firmly clamped together. The PVC tubing had no effect on algal growth. Earthworm cast material (1g) was pipetted in a slurry into a diffusion cell (Fig. 8.1)

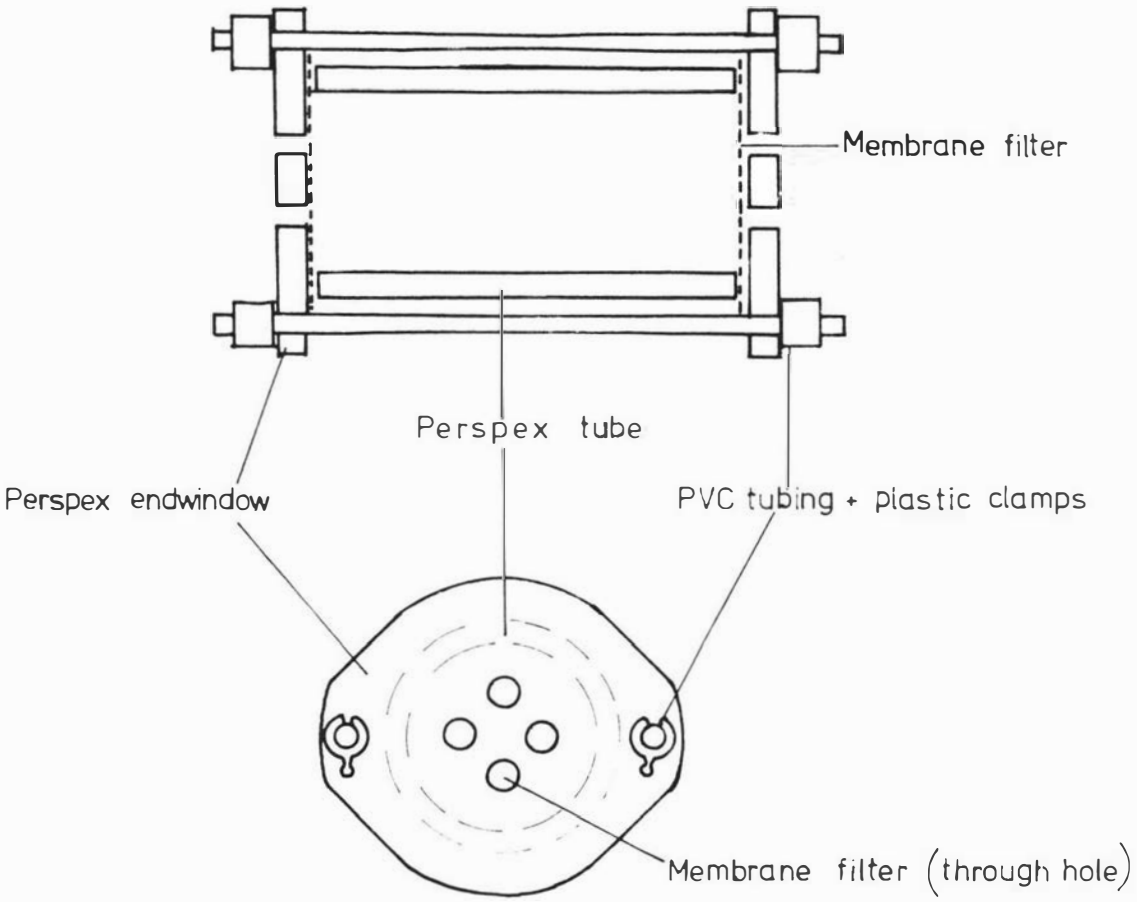

Fig. 8.1 Design of diffusion cell.

which was clamped firmly to a bench with the lower membrane filter and end-window in position but the uppermost end-window removed. The 'Perspex' tubing was filled up to the brim with ASM-1, P-, N- media, pH 7, before the second membrane filter was carefully placed over the tube end, ensuring the absence of any air bubbles. By pulling firmly on the second, 'Perspex' end-window the PVC tubing, connecting it to the end-window already in place, was stretched allowing the second end-window to pass over the end of the perspex tubing and be dropped into place where it firmly clamped the second Millipore filter. Two duplicate diffusion cells were prepared. The diffusion cells were then suspended in 1-litre of ASM-1, P-, N- growth media, pH 7. The unassembled components of a diffusion cell and 1g of earthworm casts were also added to two other culture flasks containing growth media. After a shaking for 24hr, at 28^{o}C in the dark, all cultures including a P- control were inoculated with P-starved algae (0.1M NaOH-extractable P = 0.128 µg P µg^{-1}chl). The cultures were incubated under routine liquid culture conditions. Chlorophyll concentration and alkaline phosphatase activity were monitored in all flasks. Algal 0.1M NaOH-extractable TP was monitored in the assembled diffusion-cell cultures only.

8.2.3 Solution P concentration studies

The amounts of P added to Tokomaru soil material are described in Section 8.3.3. The routine bioassay procedure described in Section 3 was used. The extractable P content of the P-starved algal inoculum was 0.204 µg 0.1M NaOH-P µg^{-1}chl.

The same procedure was used for the bioassay of the earthworm cast material, from which solution P had been removed by a series of 1-hr water extractions (soil:solution, 1:40) on an end-over-end shaker at 24^{o}C.

The extractable P content of the P-starved algal inoculum used for the bioassay was 0.130 μg 0.1M NaOH-P μg^{-1}chl.

8.2.4 Sterilization of Tokomaru soil material

Four culture flasks containing 1g of Tokomaru soil material in 900ml of distilled water were prepared. Two flasks were sterilized by autoclaving at 120°C for two periods of 15min, 1 day apart. A 100-ml aliquot of sterile ASM-1, P-, N- stock media (x 10 concentrations), pH 7, was added to all flasks. Solution IP and 0.1M NaOH-extractable IP and TP were then determined before the cultures were pre-incubated at 28°C and inoculated with P-starved algae (0.2 μg 0.1M NaOH-P μg^{-1}chl). Chlorophyll concentration was monitored over the algal growth period; sterile pipettes were used to sample the sterilized flasks.

8.3 Results and Discussion

8.3.1 Effect of algal-extracellular products on the desorption of soil phosphorus

During the 7-day incubation period, 0.1M NaOH-extractable soil OP increased in all soil systems (Table 8.1). A certain proportion of the decrease in 0.1M NaOH-extractable soil IP, therefore, was assumed tc be the result of microbial conversion of soil IP to microbial OP. Greater increases in OP occurred in the Manawatu soil systems than in the equivalent Okaihau systems; this was probably due to the larger quantities of available IP in the Manawatu systems. When filtered algal-culture media was added to the Manawatu and Okaihau systems, noticeably lower amounts of microbial OP were produced; this suggests that algal extracellular products had inhibited the rate of microbial OP production. Murphy et al. (1976) have reported that blue-green algae can produce hydroxamate chelators,

Table 8.1 Amounts of solution IP and 0.1M NaOH-extractable IP and TP, organic P increase, and pH for Manawatu and Okaihau soils (both < 30μm) as influenced by the culture media (M), the algal culture filtrate (C) in the presence and absence of Fe gel

System and (number)	Solution IP ($\mu g\ g^{-1}$) at			0.1M NaOH-extractable P ($\mu g\ g^{-1}$)						Organic P increase[1]	Final pH
				IP			TP				
	Day 1	Day 7	Difference	Day 1	Day 7	Difference	Day 1	Day 7	Difference		
					Manawatu soil						
Soil in M (1)	41	38	- 3	798	775	-23	921	927	- 6	29	6.0
Soil + Fe gel in M (2)	36	5	-31	798	579	-219	933	754	-179	40	6.1
Soil + Fe gel in C (3)	31	5	-26	793	558	-235	950	738	-212	23	6.2
Soil in C (4)	33	37	+ 4	787	751	-36	904	887	-17	19	6.2
					Okaihau soil						
Soil in M (1)	6	2	- 4	66	54	-12	150	150	0	12	5.8
Soil in Fe gel in M (2)	< 1	< 1	0	68	58	-10	151	144	- 7	3	6.3
Soil + Fe gel in C (3)	< 1	< 1	0	73	56	-17	152	146	- 6	11	6.5
Soil in C (4)	< 1	< 1	0	68	60	- 8	151	147	- 4	4	6.0

[1] Calculated from the difference between TP at days 1 and 7, and IP at days 1 and 7.

which can inhibit the growth of other species.

From the results in Table 8.1 it can be calculated that 27% of the 0.1M NaOH-extractable IP in Manawatu soil material would be made available for assimilation by algae through the simple desorption of IP from the soil. The role played by the dialysis tubing containing Fe gel is clearly illustrated, because 210 µg IP was removed from the soil solution and soil by sorption by the Fe gel. In the absence of Fe-gel the system remained in equilibrium with only a small conversion of IP to microbial OP. Consequently, the Fe gel plays a similar role to the algal cell; both are able to deplete solution IP to non-detectable levels. Over the 7-day incubation period, pH values in all systems decreased by only 0.75 - 1.0 of a pH unit; this rather small pH change was considered to have little effect on the desorption of IP from the soils.

The poorly-fertilized Okaihau soil lost little IP through desorption, only 13 µg P being sorbed by the Fe gel. On the addition of Fe gel, the already low solution IP concentration sustained by the Okaihau soil was reduced to a non-detectable level.

In the Manawatu systems with added, filtered, algal-culture media, 238 µg IP was desorbed from the soil. This value was higher than the amount of IP desorbed in the absence of soluble, algal-extracellular products (i.e., by fresh media). In the Manawatu control system (4), the addition of filtered, algal-culture media resulted in a decrease in solution IP concentration; this suggested that solution IP was converted to a form, which would not pass through the membrane filter or which would not react with the molybdate reagent in the subsequent P determination. Fogg and Westlake (1955) have shown that a soluble polypeptide produced by Anabaena can complex with phosphate ions. Alternatively, algal products could have interfered with the formation of the phosphmolybdate complex.

Similar trends were observed in the Okaihau systems. Because of the effect filtered, algal-culture media appeared to have on solution IP concentration, it was difficult to ascertain whether soluble, algal-extracellular products had any effect on the desorption of soil IP. After the appropriate corrections had been made, using results from control system (4), the addition of filtered, algal-culture media to a suspension of Manawatu soil material increased desorption by only 8%, which was not considered to be a significant change under the experimental conditions used. Filtered, algal-culture media had no effect on the desorption of IP from the Okaihau soil under these experimental conditions.

These results indicate that the desorption of IP from a well-fertilized soil plays an important role in the overall biological availability of soil IP. The fact that a large amount of IP which had previously been shown to be used by algae (Section 6), was not desorbed from Manawatu soil material and that very little IP was desorbed from the Okaihau soil material, suggest that the availability of soil IP is not governed solely by simple desorption. The data suggest that soil IP is desorbed from the soil by algal-controlled mechanisms. Because of the increase in extractable OP in all systems, it could not be determined whether there was any desorption of soil OP or any effect of algal-extracellular products on the desorption of soil OP. The algal-extracellular products present in the algal culture media appeared to have little influence on the desorption of soil IP in this experimental system. Because algae were not present in the soil-culture media suspension and were not able to maintain the concentration of any chelating agents or extracellular enzymes, any extended desorption of soil P could not be achieved. It is possible that any chelating agents and enzymes, added in the filtrate at day 1, were immediately sorbed by

soil particles, possibly releasing small amounts of soil P and then having no further effect. To evaluate this further, the two soil materials previously studied and the Tokomaru soil material were extracted with freshly-filtered media from a P-starved algal culture and the amount of IP desorbed in three consecutive 17-hr extractions was compared to the amount of IP desorbed in ASM-1, P-, N- media. The results are shown in Table 8.2. During the 17-hr extraction, the alkaline phosphatase activity of the filtrate decreased by approximately 30% in both the Manawatu and Tokomaru systems, and by approximately 60% in the Okaihau systems. This decrease in enzyme activity was assumed to be the result of sorption of the enzyme by the soil. This confirmed the suggestion that the enzyme activity in a filtered extract added to soil would not have a sustained effect over an incubation period of 7 days.

More IP was extracted from all soils by the filtered, algal-culture media than by the fresh ASM-1 media, although this was only marginally so in the case of the Tokomaru and Okaihau soil materials. Consequently, it appears probable that soluble, algal-extracellular products, including alkaline phosphatase, were responsible for the increased release of P from the soil materials.

8.3.2 Importance of algal cell-soil contact to soil phosphorus availability

The fact that soluble, algal-extracellular products caused only a small increase in the amount of P released from the soil materials studied, and that the total amount of P released was only a small fraction of biologically-available soil P (Section 8.3.1) suggest that the combined presence of the soil and algae, if not an intimate contact between the two, is required before the maximum amount of soil P can be used by the algae.

Table 8.2 Effect of algal culture media and algal culture filtrate on the release of IP from <30- μm material from three soils at a soil:solution ratio of 1:500

Material	Amount of IP (μg g^{-1}) released[1]	
	Algal culture media[2]	Algal culture filtrate[2]
Manawatu	134	147
	139	152
Tokomaru	5	8
	4	7
Okaihau	2	4
	3	4

[1] Total amount of IP extracted in three consecutive extractions.

[2] Adjusted to pH 7.

An experimental system was devised to assess the importance of algal-soil contact in relation to the amount of soil P found to be biologically available. The apparatus and culture procedures are described in Section 8.2.2. Millipore filters (< 0.45 μm), rather than dialysis tubing, were used to enclose the particulate P source material (<30- μm material from earthworm casts), because their larger pore size would probably allow algal-extracellular products, such as alkaline phosphatase, to pass from the algal culture media through to the earthworm cast material. Dialysis membrane, which is often used for the removal of inorganic cations from enzyme preparations would probably not have allowed such diffusion.

The results are presented in Fig. 8.2. The chl concentrations produced by, and the alkaline phosphatase activity of, the individual cultures indicate the relative P availability in each flask. The lack of growth (chl production) and high alkaline phosphatase activity in the P-, control culture confirm that the chl concentration and alkaline phosphatase data directly reflected the availability of the external P source, rather than internal algal P stores. The enclosure of the earthworm cast material by membrane filters greatly reduced the availability of the particulate P to the algae although a small amount of P was released to the algae over the first 11 days of the culture. The rate of P release to the algae was probably limited by the desorption of P from the casts because it had been shown previously that there was rapid diffusion of P through the membrane filters. The fact that 0.1M NaOH-algal-TP increased by 22 μg after 4 days and by 52 μg after 11 days, clearly illustrates that P was able to diffuse through the membrane filters. At 12 and 13 days the membrane filters in the duplicate flasks ruptured, allowing contact between the cast material and the algae; an immediate increase in chl

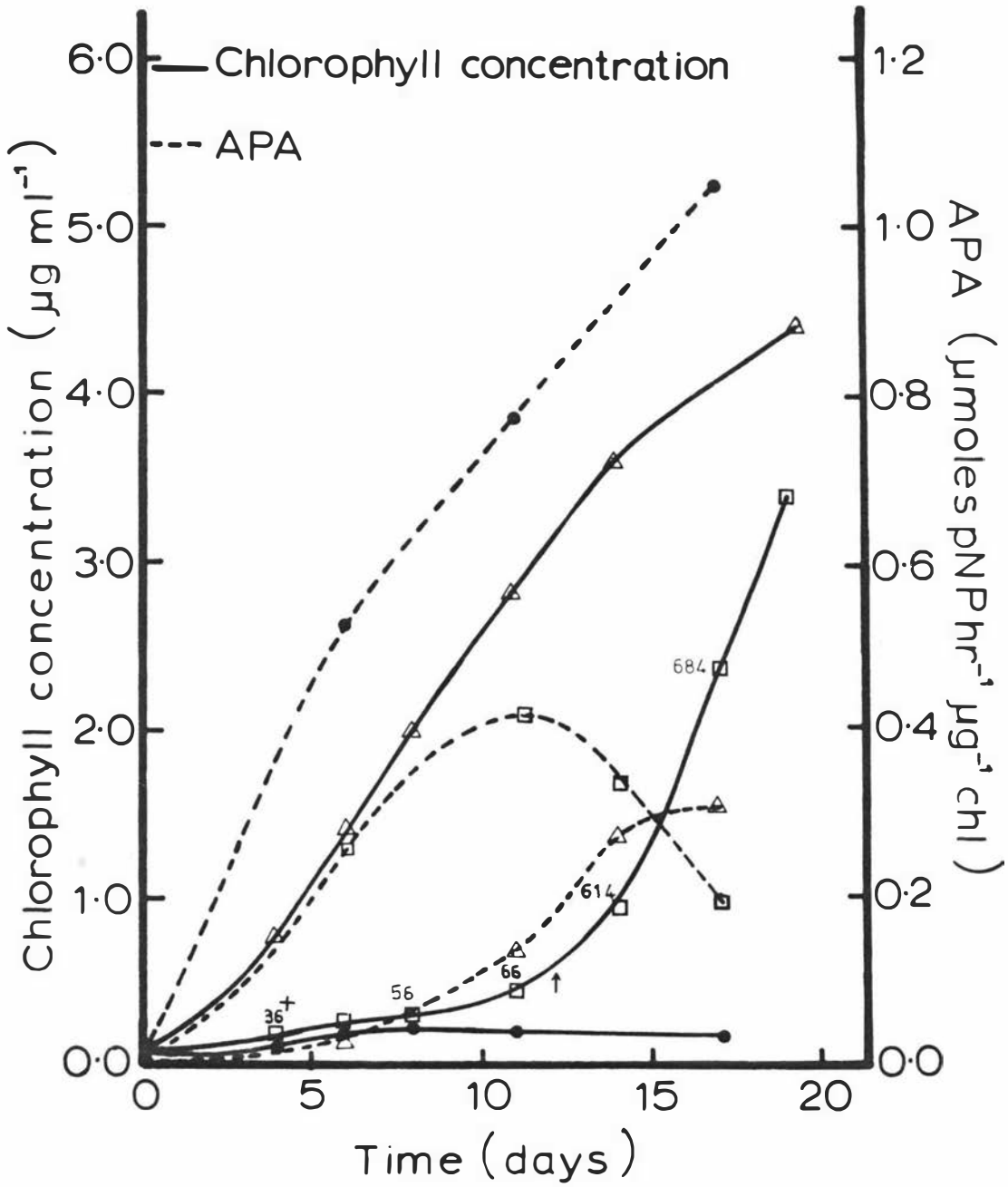

Fig. 8.2 Chlorophyll concentration and alkaline phosphatase activity (APA) during the growth of Anabaena on earthworm-cast material (△), earthworm-cast material enclosed in a permeable membrane (□), and in a P-control system (●). The amount of 0.1M NaOH-algal-TP^+ is given for the system containing earthworm-cast material enclosed in a permeable membrane, and the arrow indicates the point at which the membrane ruptured.

concentration occurred with a simultaneous decrease in alkaline phosphatase activity. This initial increase in chl concentration was followed by a more rapid increase in algal growth, which confirmed that no effect other than the separation of the algae from the cast material had prevented the utilization of earthworm cast P by algae. The reason for the rupture of the membrane filters is not clear. It appears that movement of the unclamped central area of the filter had caused the filter to tear at the clamped edge. The period during which the filter was intact (12 days) was sufficient for P diffusion to have occurred. The rupture of the filter, however, did not adversely affect the experimental results. The results show that a maximum amount of particulate P is biologically available only when there is contact between the algae and the soil.

8.3.3 Effect of solution phosphorus concentration on the availability of particulate phase phosphorus to algae

Examination of the bioassay data from Sections 5 and 6 indicate that, in general, algae which had or were initially presented with a readily-available P supply (i.e., high P content of the algal inoculum or the particulate P source maintained a relatively high solution P concentration) were capable of eventually depleting more soil P than were more severely P-starved algae. To investigate this further, two bioassays were carried out. Soil material from Tokomaru silt loam, a poorly-fertilized soil, was equilibrated in ASM-1, P-, N- media with 3 different P additions (50, 100, and 150 $\mu g\ P\ l^{-1}$) for 24hr at $28^{o}C$, and was then subjected to the routine bioassay procedure.

Tokomaru soil material sorbed most of the P added to each culture (Fig. 8.3); the addition of 50, 100, and 150 $\mu g\ P\ l^{-1}$ resulted in solution IP concentrations of 21, 40, and 68 $\mu g\ P\ l^{-1}$, respectively.

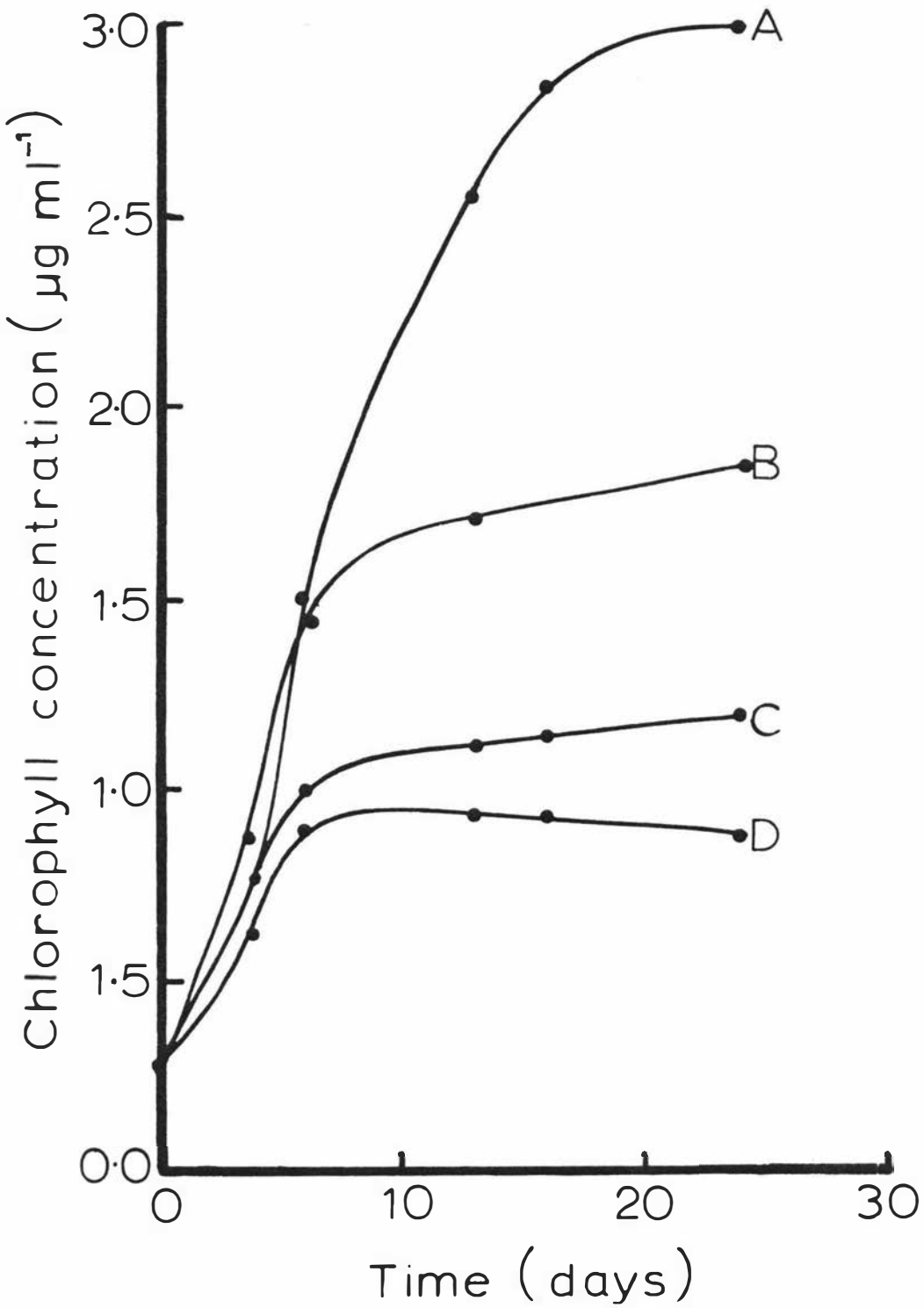

Fig. 8.3 Chlorophyll concentration during the growth of Anabaena on Tokomaru soil (<30 µm) to which P had been added (A = 150, B = 100, C = 50, and D = 0 µg P).

During the first 9 days, the rate of chl production was positively related to the solution P concentration. The increases in total algal productivity, caused by the addition of P to the soil, were not uniformly related to the amount of P added. In fact the ratio of increased algal chl:µg P added increased from 0.004 to 0.009, to 0.014 for 50, 100, and 150 µg P additions, respectively. The results suggested two possible reasons for this. Firstly, a specific amount of P was being sorbed irreversibly by the Tokomaru soil material, making it unavailable for algal growth. Consequently, the greater the amount of P added, the greater the amount which remained in the available form. Secondly, the greater and healthier algal biomass, produced by the addition of readily-available P, was capable of utilizing more soil P. Thus, the greater the amount of added P, the larger the algal production and the greater the demand on soil P. It has previously been shown (Section 7) that after 6 days of incubation, only 7% of added IP was not extracted by 0.1M NaOH from Tokomaru soil material and that 97% of 0.1M NaOH-extractable soil P was available for algal growth (Section 5). Thus, sorption of P by Tokomaru soil material would not appear to prevent most of the added IP from being used for algal growth.

From the data obtained in Section 3, it would be expected that the addition of 50, 100, and 150 µg P l^{-1} to minimally P-starved algae would produce increases in chl concentration of approximately 0.30, 0.85, and 1.42 µg ml^{-1}, respectively. In this experiment 0.20, 0.90, and 2.05 µg chl ml^{-1} were produced by additions of 50, 100 and 150 µg P l^{-1}, respectively, to the Tokomaru culture. Apart from the 50 µg P l^{-1} addition, it appears that the higher P additions resulted in additional amounts of P being made available for algal production. The source of this additional P must be the soil, which indicates that the second suggestion is probably correct.

In addition to creating a greater demand for IP, the increased concentration of hydrolytic enzymes (e.g., alkaline phosphatase) associated with the greater algal biomass (Fig. 8.3) may have been instrumental in hydrolyzing increased quantities of soil OP. Similar findings have been reported by McColl (1975), who found that the uptake of soil P by Chlorella increased when soil material was added to P solutions.

Similar trends in the relationship between the initial amount of readily-available P and total algal growth were observed in the bioassay data for earthworm cast material, from which varying amounts of P had been removed by water extraction (Fig. 8.4). Ryden and Syers (1977b) have shown that two consecutive extractions with water, each of 1hr duration, remove only that soil P which is more-physically sorbed and which shows 100% isotopic exchangeability during 30 min. Consequently, water extractions can be considered to remove a form of P from the earthworm cast material which would normally be readily available for algal growth and probably rapidly assimilated by the Anabaena during the initial stages of a bioassay.

A greater decrease in total algal growth per unit of solution P removed was observed (Fig. 8.4) as the amount of P removed from the soil increased (e.g., depletion of the readily-available soil P by 20 and 33 µg P caused biomass decreases of 0.54 and 1.20 µg chl ml^{-1}, respectively). The results suggested that a decrease in the amount of readily-available soil P resulted in less total soil P becoming available for algal growth, and also indicated that the greater the reduction of readily-available P, the greater the decrease in the amount of soil P that became available for algal growth. The initial availability of P to the algae (plotted as the reciprocal of alkaline phosphatase activity, Fig. 8.4) was much lower after water-extractable P had been removed from the earthworm casts (Fig.8.4);

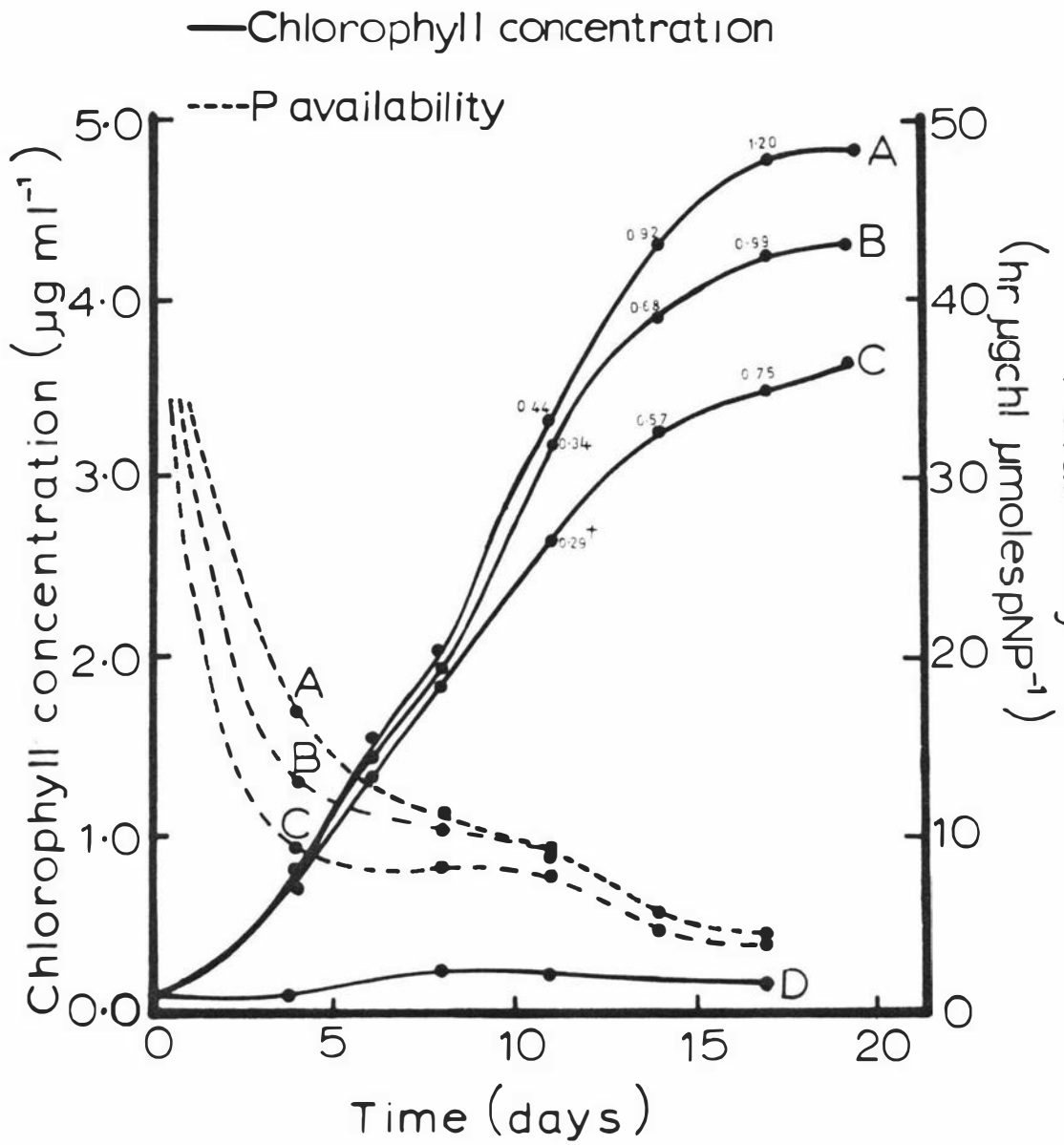

Fig. 8.4 Chlorophyll concentration, concentration of alkaline phosphatase activity^{+} (µ moles pNP $hr^{-1}ml^{-1}$), and P availability (reciprocal of alkaline phosphatase activity) during the growth of Anabaena on earthworm-cast material (<30 µm) from which varying amounts of water-extractable P had been removed (A = 0, B = 20. and C = 33 µg P).

as more water-extractable P was removed so P availability decreased. The cultures exhibiting higher initial P availability produced larger amounts of algal biomass in the early stages of the incubation, which resulted in considerable differences in algal biomass and in the concentration of alkaline phosphatase activity by day 11. From day 11 onwards, the differences in algal biomass and in the concentration of enzyme activity between the three cultures increased. It appears that the greater the algal biomass and alkaline phosphatase concentration in the latter stages of growth, the greater the amount of P that can be removed from the soil by desorption of IP and possibly by hydrolysis of soil OP. Other hydrolytic enzymes and chelating agents would also have higher concentrations at higher levels of algal population and thus would be more effective in the depletion and the utilization of soil P. The work of Miyachi et al. (1965) indicates that Chlorella elipsodea is capable of deacylating phospholipids and using the released P. It appears, however, that the relative availability of soil P to algae is not increased; only the amounts of P depleted from the soil are increased. This point is made clear by the P availability data (Fig. 8.4), which indicate that during the latter stages of growth, P availability to the algal population was very similar in all flasks.

8.3.4 Algal growth on sterile soil

To study the possible role played by bacteria and other soil microorganisms in the availability of particulate P to algae, sterilized and non-sterilized samples of Tokomaru soil material were bioassayed. The results are shown in Fig. 8.5. Unfortunately, the sterilization procedure increased the concentration of solution IP maintained by the soil material (Table 8.3), but decreased the amounts of 0.1M NaOH-soil-IP and -OP (TP - IP), the largest decrease being obtained with the OP fraction.

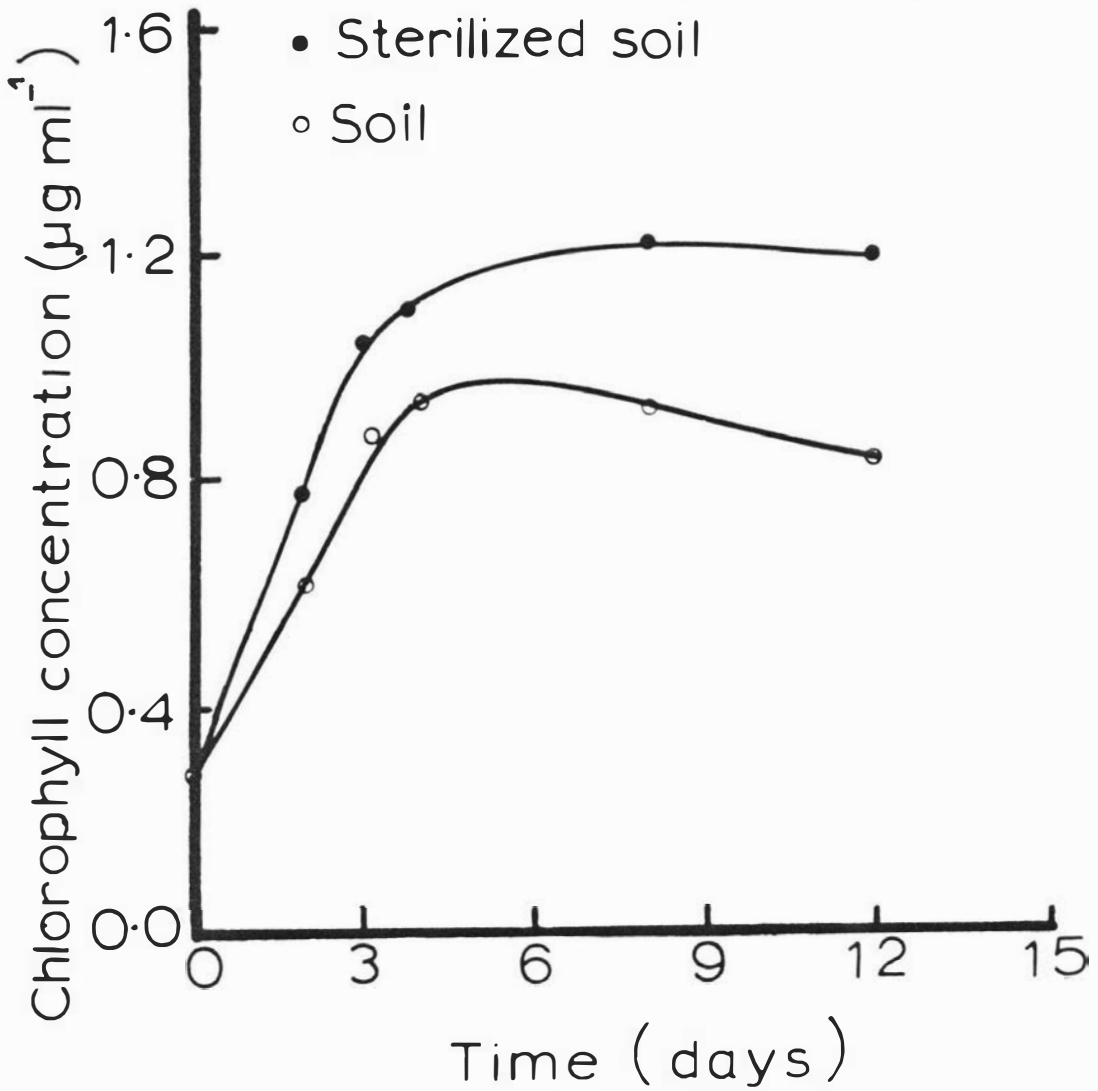

Fig. 8.5 Chlorophyll concentration during the growth of Anabaena on sterilized and non-sterilized Tokomaru soil (<30 µm).

Table 8.3 Effect of sterilization on solution IP and 0.1M NaOH-extractable P in Tokomaru soil material

	Extractable P ($\mu g\ g^{-1}$)		
	Solution IP	0.1M NaOH	
Treatment		IP	TP
Non-sterile soil	6	110	261
Sterilized soil	30	106	246

It appears that autoclaving caused the hydrolysis of some soil OP. This result contrasts with the findings of Filip and Middlebrooks (1975) who reported that solution IP decreased in autoclaved samples of pond water and feed-lot runoff and suggested that this occurred because of the formation of insoluble salts.

Because of the above results, the observed increase in algal biomass obtained with the sterilized soil (Fig. 8.5) could not be attributed solely to the removal of microbial competition for soil P. The increase in solution IP may have been responsible for the increased algal production as explained in Section 8.3.3. The small increases in algal biomass produced by lake muds, which were sterilized by UV radiation (Golterman et al., 1969) suggest that algae normally have to compete for available sediment P, rather than being dependent on bacteria for the mineralization of sediment P. Filip and Middlebrooks (1975), however, showed that in pond water and feedlot runoff, sterilization by UV radiation increased the solution P concentration. If a similar effect occurred with lake muds, then the increase in algal biomass could be accounted for by the increase in solution P.

8.4 General Discussion

Soluble, algal-extracellular products were able to increase the desorption of small amounts of particulate-phase P, presumably by the action of either chelating agents or hydrolytic enzymes. This effect, however, appears to be negligible unless high concentrations of such soluble products can be maintained. The ability of Anabaena to produce an extracellular hydroxamate chelator capable of chelating Fe, and extracellular peptides which complex Fe and P, has been reported by Murphy et al. (1976) and Fogg and Westlake (1955), respectively. If present in the culture media, these compounds would increase the release of soil P to solution. Simple phosphomonoesters may also have been hydrolyzed by alkaline phosphatase (Fitzgerald, 1966; Heath and Cook, 1975). Although Herbes et al. (1975) showed that alkaline phosphatase did not release any IP from soluble OP compounds in "concentrated" lake water, substrates for alkaline phosphatase are likely to have already been rapidly degraded or removed from solution by sorption on particulates. Consequently, this is not considered as evidence against the effectiveness of alkaline phosphatase. A lack of substrates, however, may reduce the ecological significance of alkaline phosphatase produced by algae. Heath and Cooke (1975) reported that soluble phosphomonoesters were present in lake water in the parts per billion range. While alkaline phosphatase may have limited effect on soil OP hydrolysis, blue-green algae are capable of producing several different phosphatases and other hydrolytic enzymes, as shown by their ability to use several different OP compounds as P sources (Reichardt, 1971; Stewart and Alexander, 1971).

By far the most important factor governing the availability of particulate-phase P to algae was algal contact with the particulate material.

Contact increased the depletion of P from earthworm cast material by a factor of approximately nine. It is possible that the high concentration of chelating agents and hydrolytic enxymes, which is considered to be required to effect soil P depletion, can be maintained when there is intimate contact between the algae and the particulate material. Fitzgerald (1970) has demonstrated that lake muds contained in dialysis tubing were not able to act as P sources for algal growth. Fitzgerald concluded that these lake muds were not a readily available source of P. Their inability to supply P for algal growth, however, would appear to be partly a result of the conditions imposed by the dialysis tubing (Golterman, 1973a), rather than by the phosphate chemistry of the lake sediments. Sagher (1974) has also shown that muds taken from the same lakes as those studied by Fitzgerald (1970) supported algal growth when not enclosed in dialysis tubing. For bioassays which require the separation of P source and algae, membrane filters may provide enclosures which allow greater permeability than dialysis tubing. Earthworm casts obviously sustained a sufficiently high solution P concentration to facilitate P diffusion through the membrane filter.

The importance of algal-sediment contact for the maximum utilization of sediment P, potentially has considerable ecological significance. Only in shallow water, where turbulent mixing occurs, would there be such contact between algae and sediment in an environment allowing photosynthetic production (i.e., the photic zone). Although increased biological activity is often associated with shallow areas of lakes, which is the result of a number of factors, including light intensity and water temperature, it is possible that this is also, in part, a response to the closer association between particulate-phase nutrients and microbial life in the surface waters.

The results of Wilding et al. (1974) illustrate this point. These workers have reported that decreases in bottom sediment P concentrations in a shallow lake corresponded to periods of high biological productivity in the surface water. In deeper waters, clay-sized materials which remain suspended for longer periods of time in the photic zone may be the only significant source of P (Abbot, 1957). Goldman et al. (1973) have shown a high correlation between the extent of the sediment plume of the Upper Treckee river in Lake Tahoe (California) and biological productivity.

Because particulate P availability to algae appears to be dependent on intimate contact, there is a real possibility that algal blooms could be greatly reduced and water quality improved by the removal of suspended particulate materials. The sedimentation of particulates, however, may not be completely effective in preventing the utilization of particulate P by algae. Fitzgerald (1972) has suggested that Microcystis sp. may ascend from the nutrient rich sediment-water interface with sufficient nutrients to bloom in the surface waters.

The results obtained from the bioassays of Tokomaru soil material with added solution P, and from the bioassay of earthworm-cast material from which varying amounts of water-extractable-P had been removed, illustrate the importance of an initial, readily-available P supply to a blue-green algal population. More vigorous, P-sufficient algal growth, for reasons mentioned above, may result in the depletion of more particulate-phase P by the algae, with the eventual production of a greater amount of algal biomass. Golterman et al. (1969) have suggested that for the green alga Scenedesmus, nutrient conditions at the beginning of a culture is the main factor controlling algal growth rate. With a P-assimilating,

blue-green algae, such as the Anabaena species used in this experiment, it is to be expected that the initial solution P concentration will influence population development considerably more than the slow release of P from a particulate P source. The results suggest that the reduction of dissolved P concentration in waters will probably reduce the subsequent growth of algae which results from the utilization of particulate-phase P.

SECTION 9

CHARACTERIZATION OF BIOLOGICALLY-AVAILABLE ORGANIC PHOSPHORUS IN TWO SOILS

9.1 Introduction

9.1.1 Aims of Study

The combined bioassay-soil P fractionation procedure (Sections 5 and 6) revealed that a large proportion of soil OP extracted by 0.1M NaOH was available for algal growth under the experimental conditions employed. The results for the Tokomaru (Fig. 6.9) and Manawatu (Fig. 6.14) soils showed that there was complete utilization of 0.1M NaOH-OP in these soils by Anabaena. Consequently, the forms of soil OP extracted by 0.1M NaOH were considered to be biologically available. Any attempt to identify these forms of biologically-available OP must necessarily involve characterization of the OP compounds present in the 0.1M NaOH extract. In this Section, such an attempt is made.

Although most workers have used NaOH extraction in attempts to characterize the forms of soil OP (Section 2.2.3.2), NaOH may modify several of the soil OP compounds by hydrolysis and oxidation (Bremner, 1950), and by condensation reactions between amino compounds and aldehydes and phenolics (Tinsely and Salam, 1961). Ribonucleic acids and simple sugar phosphates are particularly susceptible to alkaline hydrolysis (Baker, 1974). Although Schnitzer and Skinner (1968) have reported that humic and fulvic acids show very little modification during alkali extraction Veinot and Thomas (1972) have shown that precipitation of the humic material from an alkaline extract can lead to an approximately 25% mineralization of the organic P. Ortiz de Sera and Schnitzer (1972) have also found that alkaline extraction partially degrades humic acids. It may be argued, however, that any extractant of soil OP would invariable modify OP compounds during removal from the soil.

Extraction with 0.1M NaOH at room temperature is considered to be one of the more mild extraction procedures that have been used (Section 2.2.3.2) and it would be expected that the compounds extracted would be little modified.

Because hydrolysis and other reactions may prevent the quantitative identification of specific soil organic P compounds in alkaline extracts it was decided to characterize the OP by separation into humic and fulvic fractions (acid insoluble and acid soluble, respectively) and to study the molecular weight fractions of each by gel chromatography. Association of inositol phosphate with the fulvic fractions was studied because Baker (1974) has shown that inositol hexaphosphate is particularly resistant to hydrolysis. Other workers (Cosgrove, 1963, 1966; Martin and Wicken, 1966; Anderson, 1967; McKercher and Anderson, 1968) have shown that inositol polyphosphates may constitute a large proportion of soil OP (Section 2).

9.1.2 Techniques

Gel chromatography has become a widely-accepted technique for the isolation, purification, and fractionation of soil organic compounds. The majority of studies have involved the use of Sephadex G gels (Posner, 1963; Ferrari and Dell'Agnola, 1963; Bulter and Ladd, 1966; Lindquvist, 1967; Schnitzer and Skinner, 1968; Ladd, 1969; Bulter and Ladd, 1969; Moyer and Thomas, 1970; Steward and Tate, 1971). Ideally, separation of the organic compounds according to molecular size should be achieved. It has been found, however, that with Sephadex gels, which carry a small negative charge, adsorption effects (Gelotte, 1960; Posner, 1963; Gjessing, 1965; Swift and Posner, 1971) due to interactions between the gel and aromatic, phenolic, and heterocyclic compounds present in humic

acids (Swift and Posner, 1971) and anion exclusion effects due to charge interactions between the gel and ionic compounds of low molecular weight (such as inositol poly-phosphates) (Steward and Tate, 1969), prevent a true fractionation according to molecular size. Swift and Posner (1971) found that by packing and eluting Sephadex G gels with tris-HCl buffer the majority of adsorption interactions were reduced so that an accurate separation of humic acid material, according to molecular size, could be achieved. Swift and Posner (1971) concluded that whereas Sephadex G gels were suitable for low and intermediate molecular weight humic acids, agarose based gels provided a better media for separation according to molecular size, in the fractionation of higher molecular weight humic acids.

Strongly alkaline eluants (pH 13) have been shown to increase the degree of exclusion of low molecular weight ionic compounds from Sephadex G-25 (Steward and Tate, 1969). Use of 0.1M alkali with the more-loosely, cross-linked dextran gel Sephadex G-50 has been found to allow the partial inclusion of inositol poly-phosphates, while more complex material remains excluded (Steward and Tate, 1971). Steward and Tate (1971) suggest that this offers a useful alternative to humic acid precipitation and hypobromite oxidation for the removal of more complex organic matter during the isolation of inositol polyphosphates.

The ionic charge of the phosphate ion of OP esters allows for their separation using anion exchange resins. Work on the separation of pure phosphate esters (Bartlett, 1959) has led to the use of two main systems; resins in the chloride form eluted with HCl and resins in the formate form eluted with formic acid or ammonium formate. Elution of the chloride resin form with HCl, however, does not allow for the separation of IP from sugar phosphates (Bartlett, 1959) but does permit sugar phosphates to be grouped and separated from nucleotides and coenzymes (Lerner and

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[1] (414ml of 1M, 2-amino-2(hydroxymethyl)-propane-1,2 diol + 50ml 1M HCl 1).

Schepartz, 1968). Using the formate resin form with formic acid as the eluant, Bartlett (1959) was able to separate sugar phosphates from each other and from IP. Improved separations of soil OP, such as inositol polyphosphates, are achieved when the OP compounds have been isolated from the more complex material present in soil extracts by precipitation as barium salts (Halstead and Anderson, 1970) prior to their conversion to free esters and addition to ion exchange resins. Whole soil extracts, however, may be added to ion exchange resins (Martin, 1964a).

Ion-exchange chromatography has been used extensively to study the forms and amounts of inositol polyphosphates in soils (Cosgrove, 1963; Anderson, 1964; Martin and Wicken, 1966; Anderson, 1967; McKercher and Anderson, 1968; Moyer and Thomas 1970; Osomoto and Wild, 1970; Anderson and Malcolm, 1974). Several combinations of resin form and eluant have been used. Gradient elution of the chloride form of resin with HCl (Cosgrove, 1963; Moyer and Thomas, 1970) involves the least number of steps and provides an effective separation of inositol polyphosphates. Osomoto and Wild (1970) have slightly modified the method of Cosgrove (1963) to obtain 7 inositol P fractions from a soil extract. Inositol polyphosphates are usually identified by the comparison of their elution position with the elution position of known inositol polyphosphates on the same resin column. The standard myo-inositol polyphophates used can be identified by the ratio of P to inositol present in a pure precipitate. Inositol content may be determined by bioassay using Schizosaccharomyces pombe, for which the myo-isomer of inositol is an essential growth factor (Norris and Darbre, 1956).

9.2 Materials and Methods

9.2.1 Soil extracts

The 0.1M NaOH extracts from the <30-μm material of Tokomaru silt loam and of Manawatu silt loam were used throughout this study. The term "soil" therefore refers to <30-μm material in this Section. The humic and fulvic fractions were immediately separated from the filtered (Millipore, <0.45 μm), 0.1M-NaOH soil extracts by the following method:

(1) The pH of the extract was adjusted to pH 2 with concentrated HCl which was stored overnight at 4°C.

(2) The precipitated humic material was separated by centrifugation (IEC UV Centrifuge, 2500 rpm for 20min) and decantation of the acid-soluble fulvic material, which was adjusted to pH 7.

(3) The humic material was redissolved in 0.1M NaOH and steps 1 and 2 repeated.

(4) The humic material was adjusted to pH 7 with 0.5M NH_4OH to give the ammonium salt, which was recovered by freeze-drying and stored at 4°C.

(5) The large volume of fulvic material was concentrated by freeze drying and the sample stored at -15°C.

9.2.2 Gel chromatography

Agar gel (Oxoid, Ionagar No.2, 12%) was prepared according to the method of Andrews (1962), and was ground using a rotating blade homogeniser to pass a 250-μm sieve. The sieved granules were thoroughly washed in distilled water and then in a carbonate-bicarbonate buffer, pH 8.5 (4.68g $NaHCO_3$ + 0.506g Na_2CO_3 l^{-1}). An agar gel column (50 x 2.5cm) was prepared by pouring agar gel granules, which were suspended in the

carbonate-bicarbonate buffer, into a vertical glass chromatographic column and allowing the buffer to flow through under gravity.

Sephadex G-10-120, G-25-80, and G-50-80 were all preswollen in the eluant to be used before the columns were established. Details of column size and eluants used are given in the presentation of results. Blue dextran and tritiated water ($^{3}H_2O$) were used to determine the void (Vo) and internal (Vi) volumes of the gel beds for the calculation of Kd values (Determann, 1968). Constant flow-rates were maintained in both Sephadex and ion-exchange chromatography by the use of a Techicon Auto-analyser pump. The IP and TP concentrations of all fractions, eluted from agar gel, Sephadex G, and ion-exchange columns, were determined in the respective eluants, as described in Section 3. The relative concentration of organic material present in the fractions from the agar gel and Sephadex G columns was determined by measuring the absorbance of the fractions at 400nm (Unicam SP 1800B Ultraviolet Spectrophotometer) (Swift and Posner, 1971).

Total Fe concentration in the fractions, obtained from agar gel and Sephadex G chromatography, were determined using a Perkin-Elmer 306 atomic absorption spectrophotometer.

9.2.2.1 Fractionation of humic material

Freeze-dried ammonium humate (0.03g) was dissolved in 2ml of carbonate-bicarbonate buffer of pH 8.3 (Swift and Posner, 1972). With the aid of a fine pipette the humate solution was carefully added to the agar gel column (50 x 2.5cm) before elution with carbonate-bicarbonate buffer. Fractions (3.5ml) were collected until the absorbance of the eluate at 400nm returned to its pre-elution value. Total Fe and P concentrations of the fractions were then determined in suitable aliquots.

9.2.2.2 Fractionation of fulvic material

Because of the high NaCl content of the concentrated fulvic extract, preliminary investigations into the elution pattern of inositol hexaphosphate in the presence of a similar NaCl concentration to the fulvic extract were carried out. The NaCl concentration present had no significant effect on the elution of inositol hexaphosphate from Sephadex G-50-80 by 0.1M NaOH. The methods of Steward and Tate (1971) were used for the elution of the concentrated fulvic extract from Sephadex G-25-80 and G-50-80, by 0.1M NaOH.

Sephadex G-10-120 was used in a later study of small molecular weight fractions collected from the elution of the fulvic extract from Sephadex G-50-80. In all cases, the sample was washed into the gel bed with the minimum amount of eluant before elution with 0.1M NaOH. Details of sample size added and fraction size collected are given in the appropriate Figure. Elution of the Sephadex columns was continued until the absorbance of the eluate had returned to its pre-elution value. Total Fe and P concentrations of the fractions were determined in suitable aliquots. The P peaks on the elution curve were then identified and the respective fractions were bulked and concentrated by partial freeze drying, for the deterimation of inositol polyphosphate concentrations in material from each peak.

9.2.3 Determination of inositol polyphosphates

Initially, inositol polyphosphates were precipitated from the fulvic extract of Tokomaru silt loam by the following method (Baker, 1977):

(1) The fulvic extract was adjusted to pH 9 with NH_4OH

(2) All P compounds were precipitated by adding 10ml of 15% $BaCl_2$ followed by 2 volumes of ethanol. The mixture was maintained at 4^oC overnight.

(3) The precipitate was removed by centrifugation at 27000 x G (Sorvall Superspeed RC2 B) and washed twice with 50% ethanol and once with absolute ethanol.

(4) Ethanol was evaporated off at room temp, leaving a slightly moist precipitate.

(5) The phosphates were then converted to their acid form by stirring the barium precipitate with Amberlite IR 120 (H+) resin in a small volume of distilled water.

(6) The solution of acid-soluble phosphates was separated from the resin by filtration (Whatman No.1 filter paper) and stored at -15°C. Aliquots of this solution were fractionated using a micro-column (10 x 0.2cm) of Dowex-AG1 x 8-200 (chloride form) and eluting with an HCl gradient.

Sodium inositol polyphosphates were prepared from the Sephadex G-50 fractions by the purification procedure of Moyer and Thomas (1970). The condensed solutions were stored at -15°C. Aliquots of these solutions were fractionated using the micro anion-exchange column procedure also described by Moyer and Thomas (1970). Details of sample size, column dimensions, and eluate fraction size are given with the results. The TP concentration of each fraction was determined. The elution pattern of the soil inositol phosphates was compared with the elution pattern of a hydrolysate of sodium inositol hexaphosphate, which was prepared from calcium inositol hexaphosphate (Sigma Chemicals) by the method of Cosgrove (1963) and hydrolyzed by the method of Desjobert and Petek (1956). Inositol polyphosphates were identified in the eluted peaks, from the fractionation of the hydrolysate, by comparison to the elution patterns obtained by Moyer and Thomas (1970) and by the inositol:P ratio in the

fraction of each peak that had the highest P concentration.

The inositol concentration was determined by the bioassay method of Norris and Darbre (1956), as modified by Paranjapye et al. (1965).

9.3 Results and Discussion

Of the OP present in the 0.1M NaOH extract from the Tokomaru soil (based on the gel-chromatography fractionation results, discussed below), 35% was humic-OP and 65% was fulvic-OP.

9.3.1 Fractionation of humic material

The elution pattern of the organic matter (absorbance at 400nm) and the Fe and P contents of the fractions collected from the agar-gel chromatography of the humic acid fraction of Tokomaru silt loam are shown in Fig. 9.1. The results indicated that there was total recovery of humic-P from the column. It is thought that the fractionation technique used here, allows continuous fractionation throughout the molecular weight distribution (Swift and Posner, 1972). By direct comparison with the work of Andrews (1962), the molecular weight of the humic material appears to range from below 10,000 to above 70,000, with the major organic matter fraction having an approximate molecular weight of 10,000. Higher TP concentrations were associated with the higher molecular weight material, which appeared as a shoulder (fractions 28 and 29) to the main peak (Fig. 9.1). The TP concentration per unit of organic material for these fractions was considerably higher than in any of the other fractions eluted. In fact TP concentration per unit of organic material decreased as the molecular weight of the humic material decreased. A similar trend was observed for the association of Fe with the organic matter (Fig. 9.1). High concentrations of Fe were only associated with the higher molecular

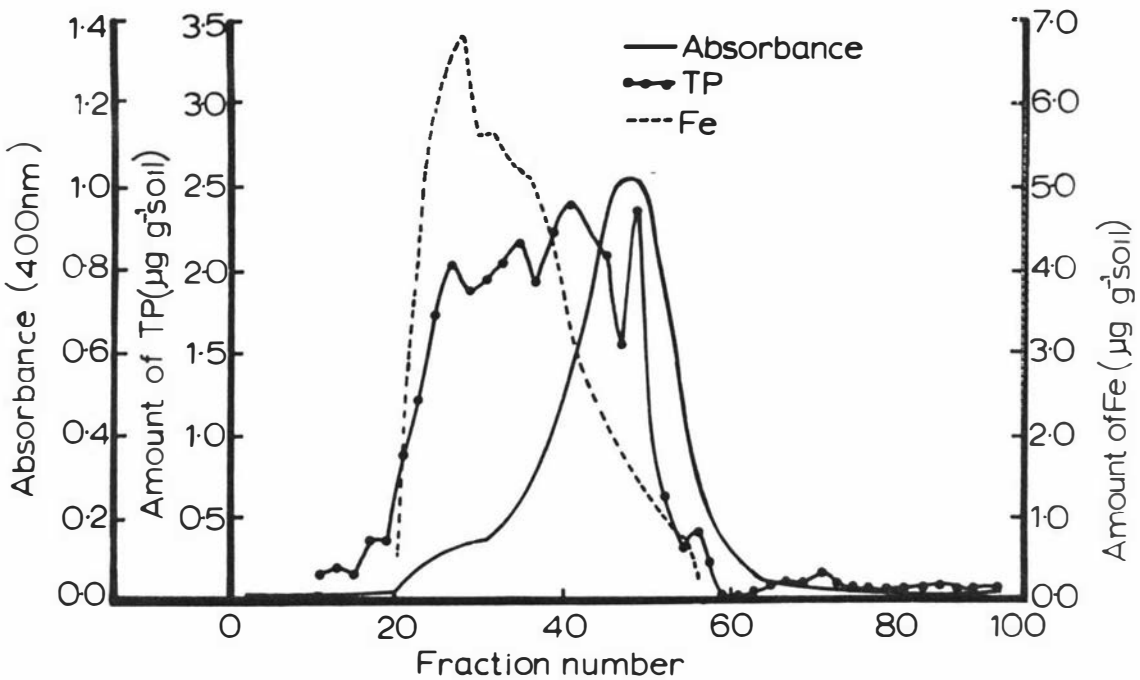

Fig. 9.1 Fractionation of the 0.1M NaOH humic extract from Tokomaru soil (<30 µm) on 12% agar gel (<250-µm bead size) using carbonate-bicarbonate buffer as the eluant (sample size = 2 ml, column = 50 x 2.5cm, and fraction volume = 3.5ml).

weight fractions. It was also noticeable that most of the TP was only associated with the organic matter fractions containing Fe. It was assumed that the Fe, organic matter, and P were part of the same complex because Fe is retained by the gel (especially under alkaline conditions) unless it is complexed with organic matter (Plumb and Lee, 1973). Although in the presence of NaOH, the hydroxoferrate ion may form (Scholder, 1965), if present it should be eluted after the organic material, because it is sufficiently small to easily diffuse into the agar gel. Consequently, the results suggest that a humic-Fe-P complex is a major form of humic-P extracted from Tokomaru soil material by 0.1M NaOH.

9.3.2 Fractionation of the fulvic extract from Tokomaru soil

9.3.2.1 Using Sephadex G-25-80

The data in Fig. 9.2 show the elution patterns of organic matter, Fe, TP, and IP obtained from the fractionation of the 0.1M-NaOH extract of the soil using Sephadex G-25. The elution volumes of dextran blue (Vo) and tritiated water (Vi + Vo) through the same column allowed the calculation of distribution coefficients (Kd) for the P peaks from their elution volume (Ve):

$$Kd = (Ve - Vo) / Vi$$

The elution patterns of organic matter and TP were closely related, although the organic matter peak (Kd = 0.83) eluted last was not associated with OP but was eluted simultaneously with the major IP fraction. Very little Fe was associated with the excluded organic matter (Kd = 0), although Fe concentration did peak slightly in fractions 8 and 9. The major Fe peak (Kd = 0.74) appeared to be associated with a small organic matter peak with which OP, but largely IP, was also eluted. Because there was partial separation of the final organic matter (Kd = 0.83) and the Fe (Kd = 0.74)

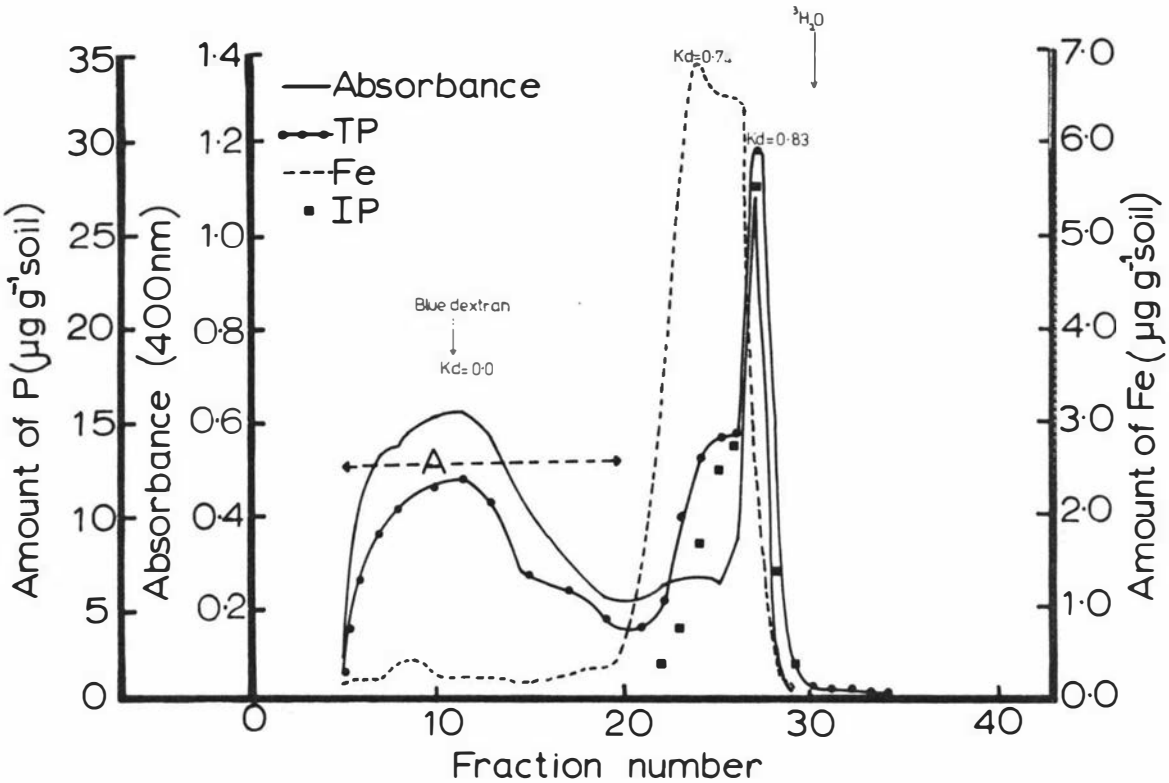

Fig. 9.2 Fractionation of the 0.1M NaOH fulvic extract from Tokomaru soil (<30 µm) on Sephadex G-25 using 0.1M NaOH as the eluant (sample size = 10 ml, column = 24 x 1.6cm, and fraction volume = 1.6ml). Fractions under A were bulked for subsequent fractionation on Sephadex G-50.

peaks, the two were not considered to be associated with each other (see Section 9.3.3.2).

The results indicate that the major form of OP present in the fulvic acid material extracted from Tokomaru silt loam is excluded from Sephadex G-25 and can therefore be considered to have a molecular weight > 5,000 (see Section 9.4). The remainder of the OP occurs as a slightly included fraction (a shoulder to the main peak, fractions 15 - 19) and small OP molecules of molecular weight ≤ 1000, that are eluted just before (Kd = 0.74) and simultaneously to IP. The OP (Kd = 0.74) appears to be associated with Fe.

9.3.2.2 Using Sephadex G-50-80

The OP fractions excluded from Sephadex G-25 and the slightly included organic P fractions (A in Fig. 9.2) were bulked, concentrated by freeze drying, and fractionated using Sephadex G-50 and 0.1M NaOH as the eluant. The elution pattern is shown in Fig. 9.3. Total recovery of the TP eluted through the column was obtained. Some organic matter was excluded from the gel (Kd 0.02); OP which probably consisted of material having a molecular weight > 30,000, was also associated with this peak. The majority of organic matter was slightly included (Kd = 0.17), suggesting a molecular weight of ≤ 30,000. This organic matter was associated with OP peaks having Kd values of 0.26 and 0.40. As with the Sephadex G-25 fractionation, the final peak of organic matter was highly coloured and was eluted with a large amount of IP and a small amount of OP. This indicated the presence of OP material of molecular weight ≤ 1000, which may have arisen partly from the breakdown of higher molecular weight OP material during the concentration steps involved in preparing the material for fractionation on Sephadex G-50.

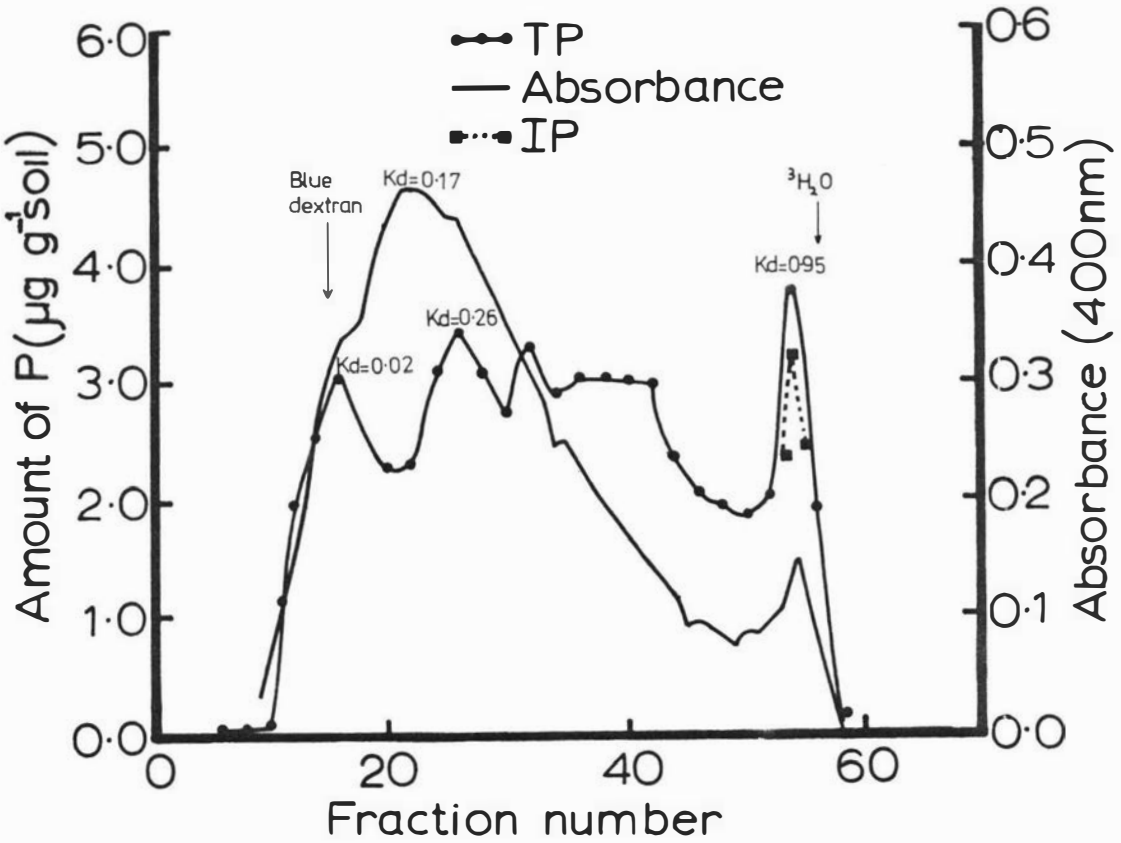

Fig. 9.3 Fractionation of the material (A), excluded from and partially included by Sephadex G-25, on Sephadex G-50 using 0.1M NaOH as the eluant (sample size = 5 ml, column = 24 x 1.6cm, and fraction volume = 0.86 ml).

The elution pattern of TP did not show a distinctive peak indicating the presence of inositol polyphosphates; this would be expected to appear with a Kd = 0.40 (Steward and Tate, 1971). A small peak did appear with Kd = 0.40 but as this only differed from the fractions in close proximity by 0.3μgPg^{-1} soil, it was not considered significant. In order to confirm the absence or presence of inositol polyphosphates in the 0.1M NaOH extract, the fulvic material, without the Sephadex G-25 pretreatment, was fractionated using a longer column of Sephadex G-50. The elution pattern is shown in Fig. 9.4. There was total recovery of TP eluted through the column. A greater separation between excluded, partially included, and totally included P forms was achieved. Also the final peak of organic matter, which in previous fractionations had been eluted with the IP, was now eluted slightly before IP. The OP material of low molecular weight was still eluted with the IP. Although greater separation of the fractions was achieved, the results suggested that only a small amount of the OP in the fulvic fraction consisted of free inositol polyphosphates, because only a small peak appeared with a Kd of 0.4.

9.3.2.3 Identification of inositol polyphosphates by ion-exchange chromatography

In order to facilitate identification of inositol polyphosphate peaks in the TP elution patterns of the Sephadex G-50 fractions eluted from the ion exchange column, inositol polyphosphates were identified in the TP elution pattern obtained from the fractionation of an inositol hexaphosphate hydrolyzate. The method is described in Section 9.2. The bioassay of inositol gave the P:inositol ratios shown in Fig. 9.5. Reliable results were only obtained when the phosphates of each fraction were first precipitated as barium phosphates before the hydrolysis and

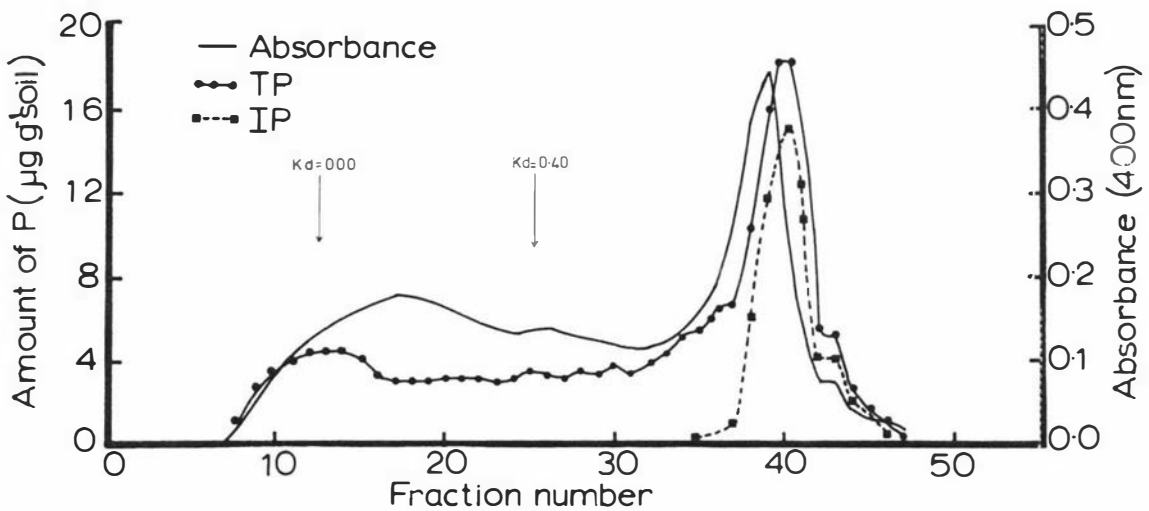

Fig. 9.4 Fractionation of the 0.1M NaOH fulvic extract from Tokomaru soil (<30 µm) on Sephadex G-50 using 0.1M NaOH as the eluant (sample size = 15 ml, column = 40 x 2.5cm, and fraction volume = 5ml).

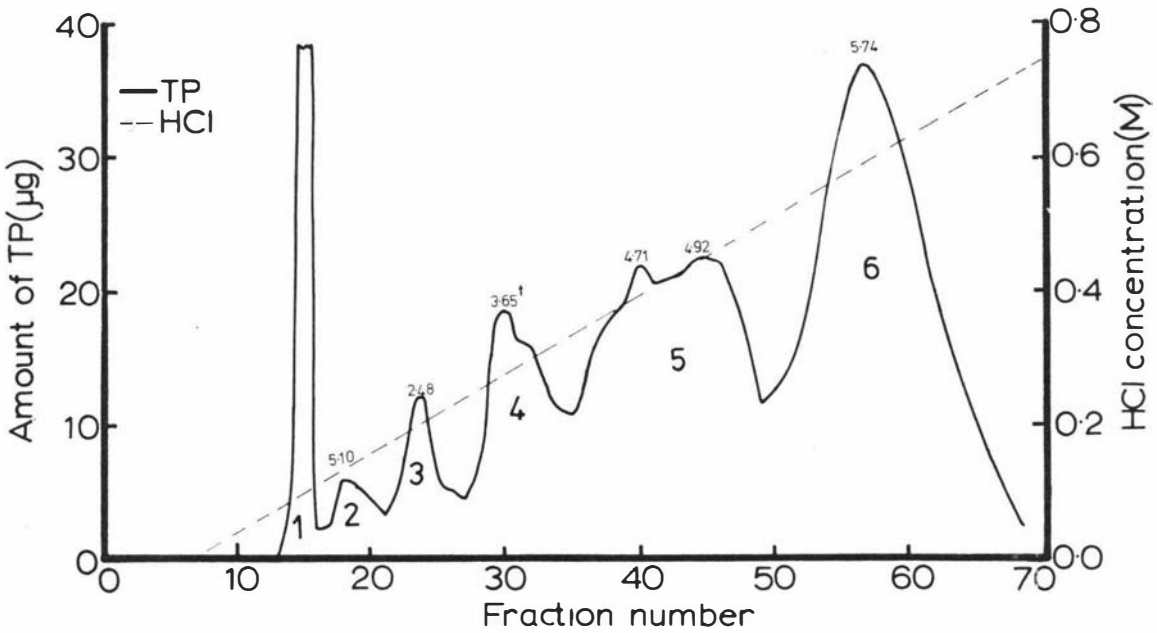

Fig. 9.5 Fractionation of inositol hexaphosphate hydrolyzate on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (— —) (sample volume = 0.4ml, column = 10 x 0.2cm, and fraction volume = 1.5ml). + = ratio of P:inositol. The peaks are numbered 1 - 6 for comparison with other elution patterns and are discussed in the text.

bioassay procedures. From the ratios obtained it was only possible to reliably identify peaks 4, 5, and 6 as inositol tetraphosphate (iP_4), inositol pentaphosphates (iP_5) (two peaks), and inositol hexaphosphate (iP_6). Peak 2 appeared to contain inositol contaminated by IP; peak 3 may have been a mixture of inositol diphosphate (iP_2) and inositol triphosphate (iP_3). Peak 1 was identified as IP from the elution of KH_2PO_4 but also appeared to contain some inositol. Osomoto and Wild (1970) have reported the presence of iP_1, iP_2, and iP_3 in this fraction.

Initially, inositol polyphosphates prepared from the whole fulvic fraction by the method of Baker (1977) were fractionated using the ion-exchange resin. The TP elution pattern is shown in Fig. 9.6. A large amount of complex OP was still present in the extract and was not adsorbed by the ion-exchange resin, being freely eluted from the column by distilled water (fractions 1 - 4). Considerable amounts of IP (peak 1) and TP (peak 2), thought to be a mixture of iP_1, iP_2, and iP_3, appeared in the eluted fractions. Only small TP peaks, equivalent to peaks 3, 4, 5, and 6 of the standard elution pattern, were distinquishable above the background levels of TP.

In order to obtain clear elution patterns, from which inositol polyphosphates could be identified, it was decided to study the higher molecular weight OP material separately from the lower molecular weight OP material and to purify the phosphate forms by the procedure of Moyer and Thomas (1970). Therefore, the fulvic OP excluded from Sephadex G-50 (fractions 7 - 22, Fig. 9.4) and fractions 23 - 37, corresponding to the theoretical position of inositol polyphosphate (Kd = 0.4), (Steward and Tate, 1971) were bulked and prepared by the method of Moyer and Thomas (1970), for ion-exchange chromatography.

The results of the ion-exchange chromatography are shown in Fig. 9.7

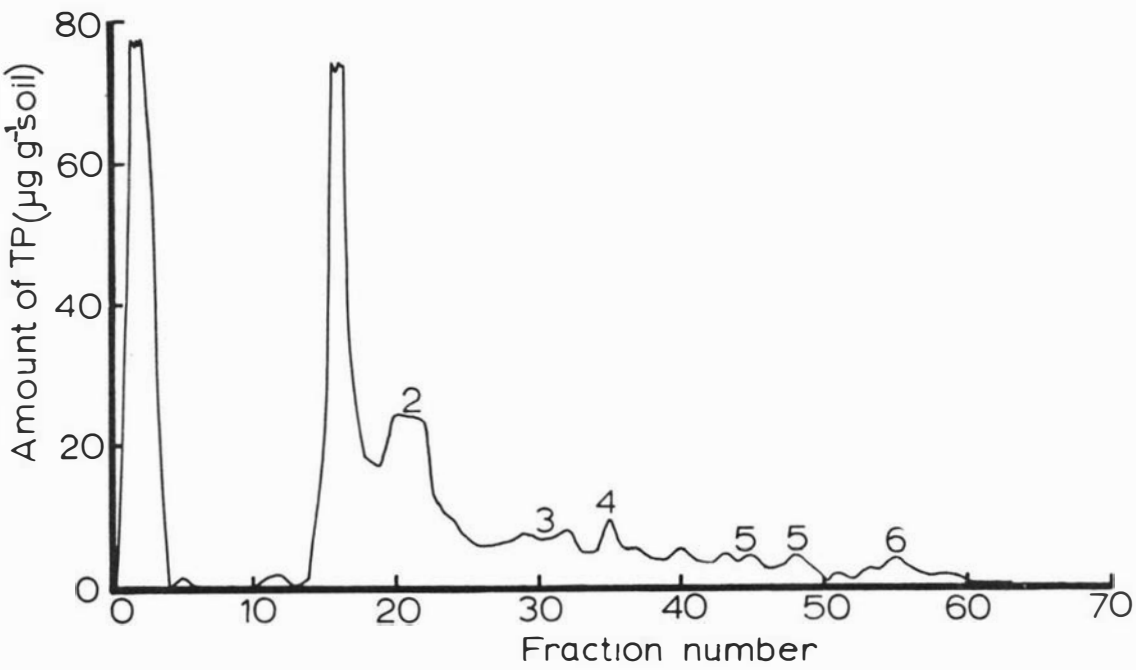

Fig. 9.6 Fractionation of inositol polyphosphates, prepared from the 0.1M NaOH fulvic extract of Tokomaru soil (<30 μm) by the method of Baker (1977), on ion exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (sample size = 1ml, column = 10 x 0.2cm, and fraction volume = 1.4ml). The numbered peaks are discussed in the text.

and Fig. 9.8. During the preparation of the samples, over 70% of the OP was hydrolyzed, hence the results are expressed as μg P per fraction, rather than $\mu g\ P\ g^{-1}$ soil.

The hydrolyzate of the OP excluded from Sephadex G-50 gave elution TP peaks (Fig. 9.7) corresponding to all the peaks identified in Fig. 9.5. Relatively more of the lower inositol P esters (peaks 2, 3, and 4) were present than iP_5 and iP_6 (peaks 5 and 6), which appeared only in trace amounts. It is probable that the purification procedure caused some hydrolysis of iP_6 (Moyer and Thomas, 1970; Osomoto and Wild, 1970), which probably resulted in the overestimation of the lower esters of inositol polyphosphate. The purification procedure was successful in removing amounts of the more complex OP because the amount of P not adsorbed by the resin was considerably reduced. The TP peak (7), eluted after the peaks assumed to be iP_6, may have been due to the scyllo-isomer of iP_6 (Moyer and Thomas, 1970; Osomoto and Wild, 1970).

The TP elution pattern obtained for the ion-exchange chromatography of Sephadex G-50, fractions 23 - 37 (Fig. 9.8) clearly shows large peaks (2 and 3), assumed to be iP_1, iP_2, and iP_3; only trace amounts of iP_4 and iP_5 were detected. Although it is probable that iP_6 exists in this fraction, the considerable hydrolysis occurring during the purification procedure suggests it is likely that iP_6 was hydrolyzed to form the lower inositol polyphosphate esters and IP. Using the same procedure, Moyer and Thomas (1970) found that the recovery of standard inositol hexaphosphate after purification was only 34%.

9.3.3 Fractionation of the fulvic extract from Manawatu soil

9.3.3.1 Using Sephadex G-50-80

The elution patterns of organic matter, TP, and IP from the

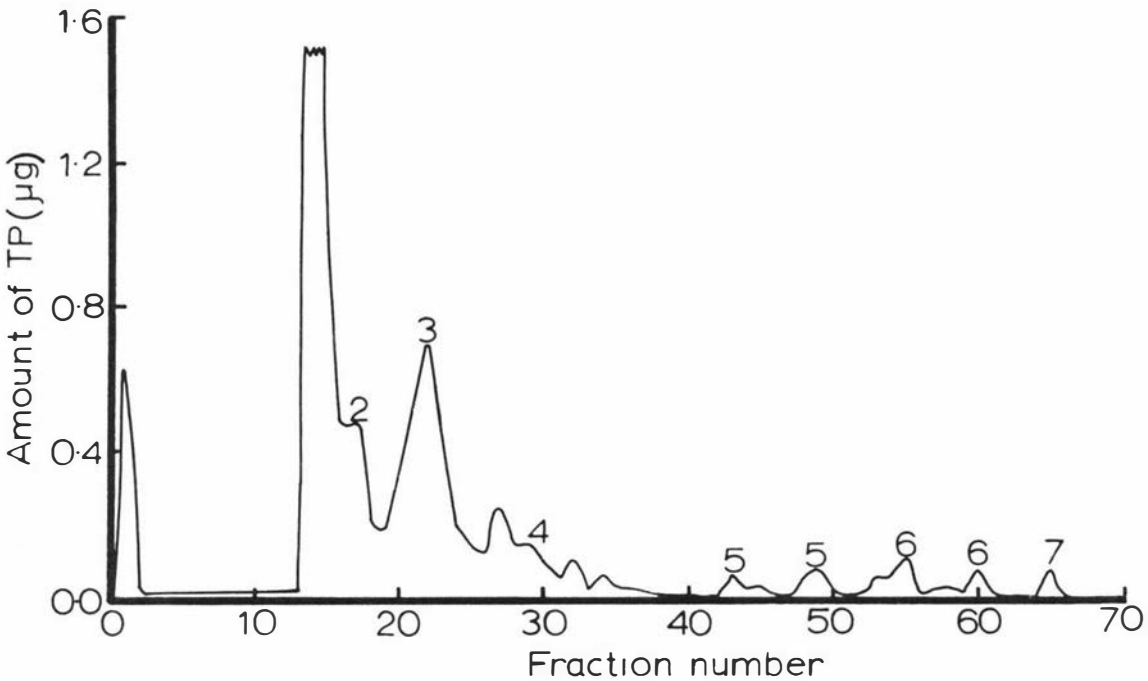

Fig. 9.7 Fractionation of inositol polyphosphates, prepared from Sephadex G-50 fractions 7 - 22 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (sample size = 1ml, column = 10 x 0.2cm, and fraction volume = 1.6ml). The numbered peaks are discussed in the text.

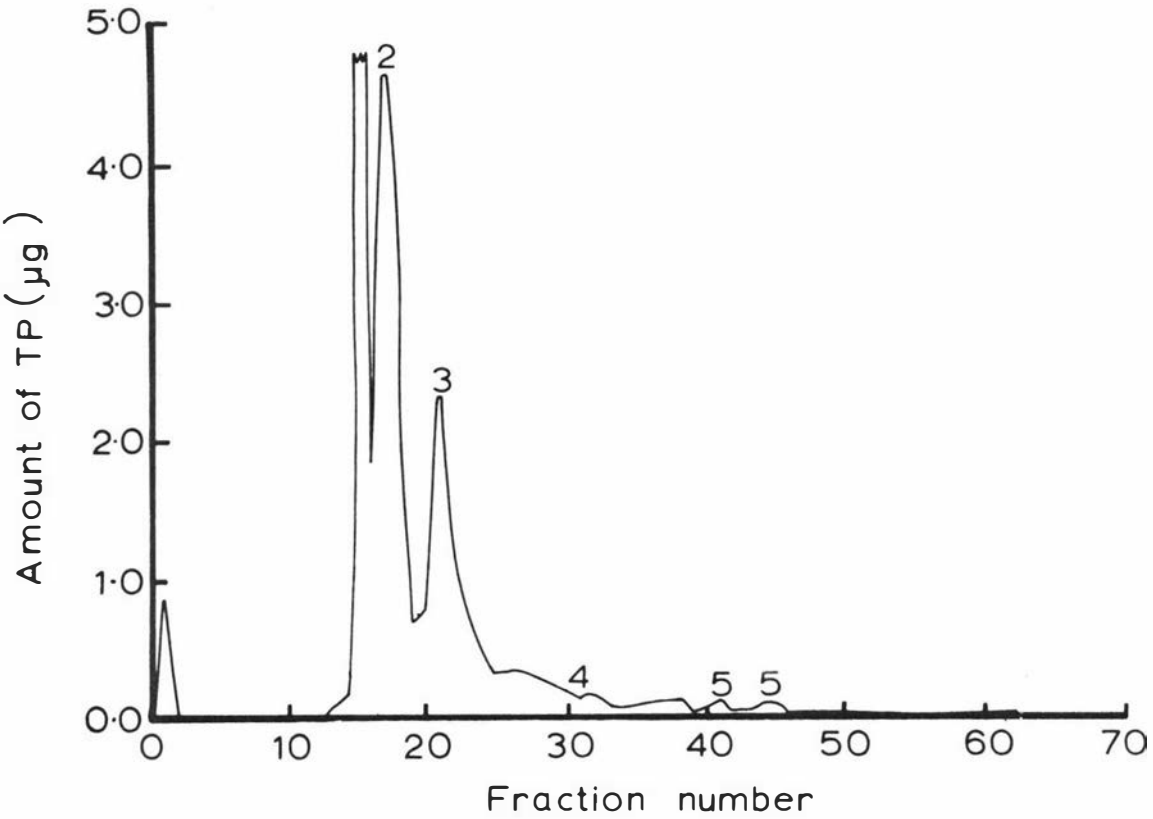

Fig. 9.8 Fractionation of inositol polyphosphates, prepared from Sephadex G-50 fractions 23 - 37 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (sample size = 1ml, column = 10 x 0.2cm, and fraction volume = 1.5ml). The numbered peaks are discussed in the text.

fractionation of the fulvic material using Sephadex G-50 and 0.1M NaOH as the elutant (Fig. 9.9) were similar to the patterns for the Tokomaru fulvic material. Much larger amounts of IP were present, however, and a significant OP peak was eluted with a Kd of 0.40; this was assumed to be inositol polyphosphate (Steward and Tate, 1971). Compared to the total amount of P present only a small amount of OP was excluded from the gel (Kd = 0), showing that the majority of OP had a molecular weight <30,000. The fractions were bulked as follows: 8 - 17, 18 - 28, and 29 - 35, reduced in volume by partial freeze drying, and purified by the method of Moyer and Thomas (1970). Aliquots of the purified fractions were eluted through columns of ion-exchange resin.

9.3.3.2 Identification of inositol polyphosphates by ion-exchange chromatography

The TP elution patterns of the purified Sephadex G-50 fractions, obtained using ion-exchange chromatography, were compared to the TP elution pattern in Fig. 9.5 in order to identify the presence of inositol polyphosphates. When the OP which had been excluded from Sephadex G-50 (fractions 8 - 17, Fig. 9.9) was eluted from the ion-exchange column, the TP elution pattern contained peaks equivalent to trace amounts of all the inositol polyphosphate esters (Fig. 9.10). A large amount of complex OP was not adsorbed by the resin and was eluted with distilled water (fractions 1 - 4, Fig. 9.10). The large IP peak (1) indicated that considerable hydrolysis of OP had occurred during the purification procedure. The elution of a TP peak (7) at fraction number 72 suggests the presence of a small amount of the scyllo-isomer of iP_6.

The TP elution pattern (Fig. 6.11) of the Sephadex G-50 fractions 18 - 28, which were thought to include free inositol polyphosphates, indicated the presence of larger amounts of all inositol polyphosphates,

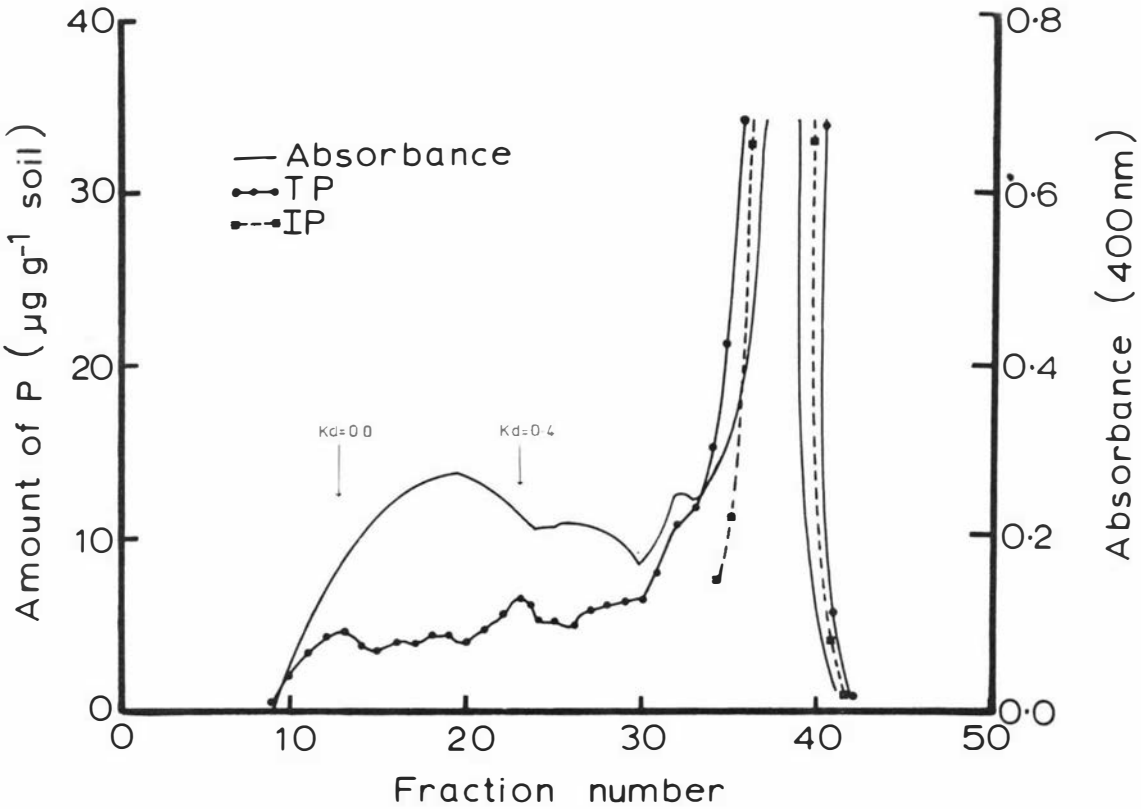

Fig. 9.9 Fractionation of the 0.1M NaOH fulvic extract from Manawatu soil (< 30 µm) on Sephadex G-50 using 0.1M NaOH as the eluant (sample size = 10ml, column = 38 x 2.5cm, and fraction volume = 4.5ml).

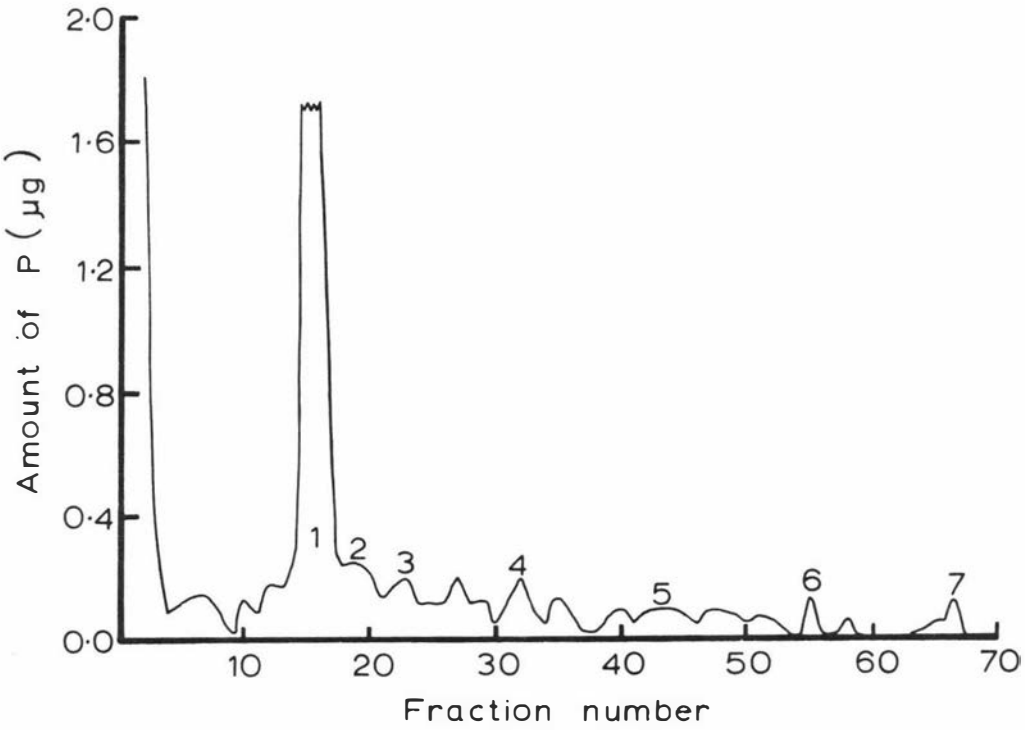

Fig. 9.10 Fractionation of inositol polyphosphates, prepared from Sephadex G-50 fractions 8 - 17 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (sample size = 2ml, column = 10 x 0.2cm, and fraction volume = 1.5 ml). The numbered peaks are discussed in the text.

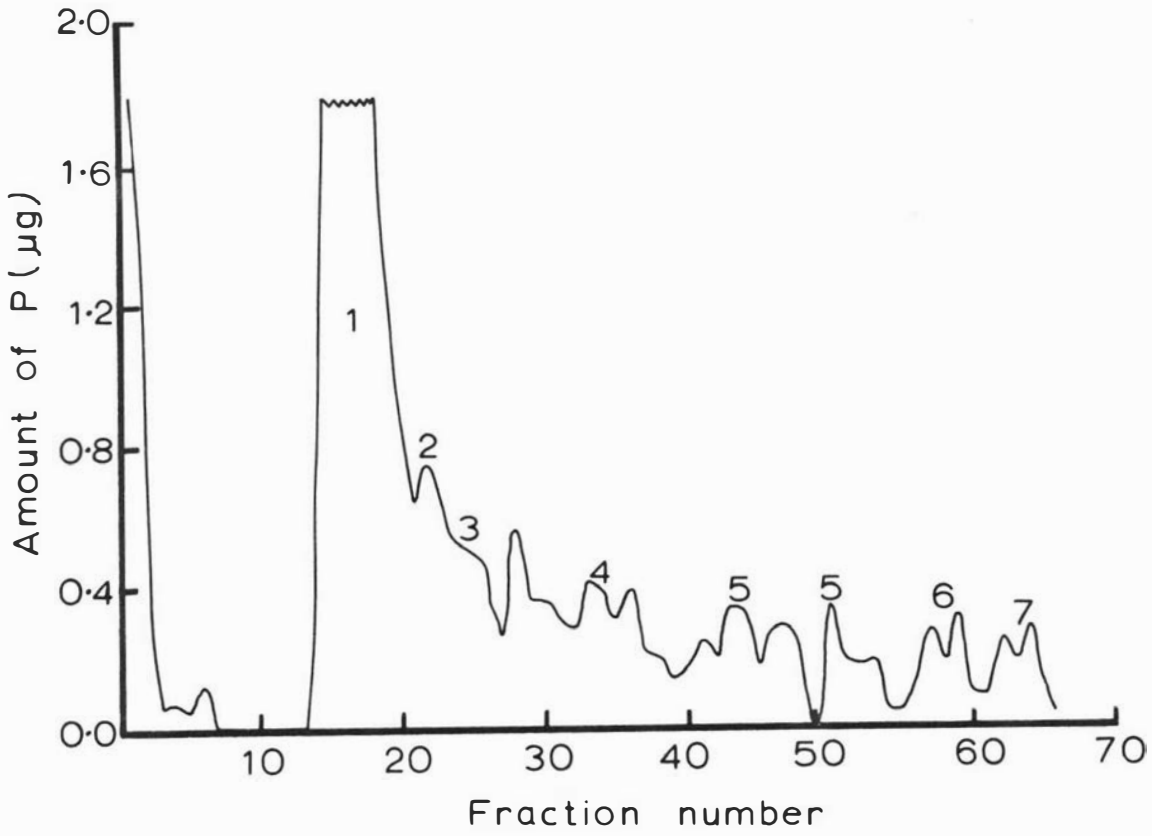

Fig. 9.11 Fractionation of inositol polyphosphates prepared from Sephadex G-50 fractions 18 - 28 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (sample size = 2ml, column = 10 x 0.2cm, and fraction volume = 1.5ml). The numbered peaks are discussed in the text.

including the scyllo-isomer of iP_6, than were present in the excluded fraction (8 - 17, Fig. 9.10). Again a large amount of OP appeared to be complexed and was not adsorbed by the resin.

The Sephadex G-50 fractions 29 - 35, containing OP of lower molecular weight than the previous Sephadex G-50 fractions, gave a TP elution pattern that indicated the presence of a small amount of complexed OP and large amounts of iP_1, iP_2, and iP_3 (peaks 2 and 3, Fig. 9.12). Smaller peaks equivalent to iP_4, iP_5, iP_6, and the scyllo-isomer of iP_6 were also present.

In summary, the TP elution patterns obtained using ion-exchange chromatography suggest that iP_6 and its scyllo-isomer and lower inositol polyphosphate esters are present in the OP fraction excluded from Sephadex G-50, in the OP fraction (Kd = 0.40) equivalent to free inositol polyphosphates, and in the OP fraction eluted from Sephadex G-50 slightly ahead of IP. The contribution made by the lower esters to the amounts of inositol polyphosphate present in each fraction is probably overestimated because it is likely that certain amounts of the lower esters originate from the hydrolysis of iP_6 during the purification procedure (Osomoto and Wild, 1970).

9.3.3.3 The association of iron with the low molecular weight organic phosphate fractions obtained using Sephadex gel chromatography

A 10-ml aliquot of the fulvic extract from the Manawatu soil was fractionated using Sephadex G-50. The elution patterns of organic matter, TP, IP, and Fe are shown in Fig. 9.13. As with the fractionation of the Tokomaru fulvic extract on Sephadex G-25 (Fig. 9.2), Fe was associated with the organic matter and OP fractions; the highest concentration of Fe, again, was eluted with the low molecular weight OP

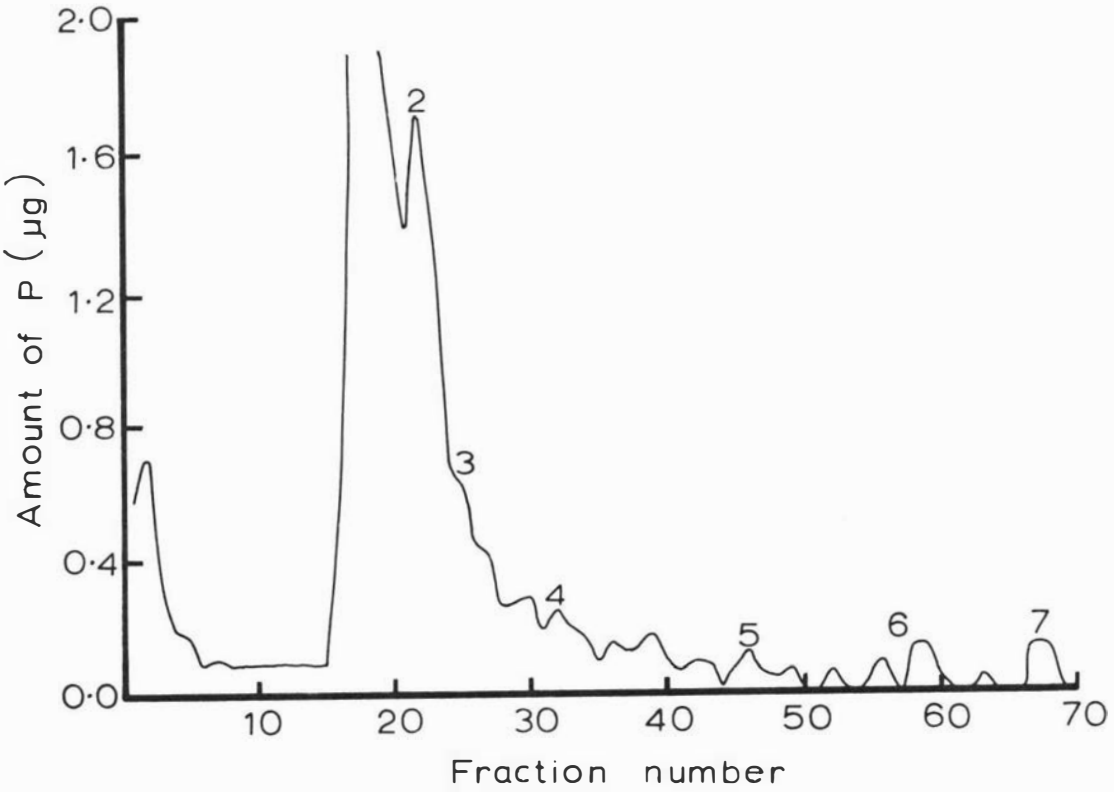

Fig. 9.12 Fractionation of inositol polyphosphates prepared from Sephadex G-50 fractions 29 - 35 by the method of Moyer and Thomas (1970) using gradient elution with HCl (sample size = 3ml, column = 10 x 0.2 cm, and fraction volume = 1.4ml). The numbered peaks are discussed in the text.

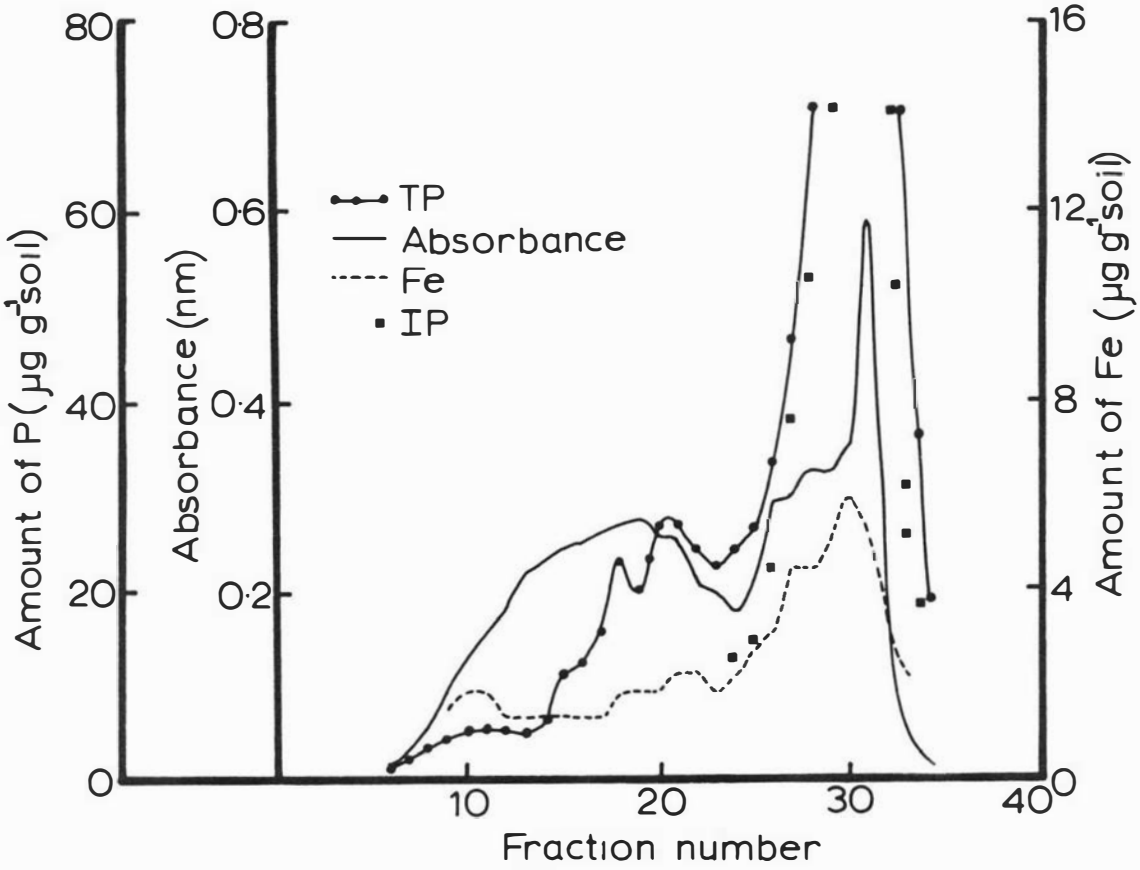

Fig. 9.13 Fractionation of the 0.1M NaOH fulvic extract from Manawatu soil (<30 µ m) on Sephadex G-50 using 0.1M NaOH as the eluant (sample size = 10ml, column = 28 x 2.5cm, and fraction volume = 5.5 ml).

and IP fractions. To determine the nature of this association of Fe with P, fractions 27 - 32 (Fig. 9.13) were bulked and an aliquot fractionated using Sephadex G-10. The elution patterns of organic matter, TP, IP, and Fe are shown in Fig. 9.14. Total recovery of TP, IP, and Fe from the gel column was obtained. The elution position of blue dextran suggests that the small OP molecules and IP in the TP peak were excluded from the gel by charge effects (Steward and Tate, 1969). Only a small amount of OP was present in the sample and this was eluted simultaneously with the IP. The Fe peak was distinct from the large amounts of organic matter and TP eluted from the column. Small amounts of Fe, however, were present in all fractions containing organic matter or P. A very small amount of OP was associated with the major Fe peak.

The results suggest that the major amount of Fe is present in a form which is not associated with organic matter, because organic matter concentration does not increase with increasing Fe concentration. The Fe, however, may be associated with small amounts of OP.

9.4 General Discussion

The approximate values obtained, by agar gel chromatography, for the molecular weight range of humic acids in the 0.1M NaOH extract from Tokomaru soil compare favourably with the molecular weight range of humic acids extracted from Australian soils (Swift et al., 1970). High molecular weight humic acids contained the highest TP concentrations and the TP content per unit weight of organic material decreased as molecular weight decreased. Swift and Posner (1972) have also shown that P contents are greatest in high molecular weight fractions and decrease considerably with decreasing humic acid molecular weight.

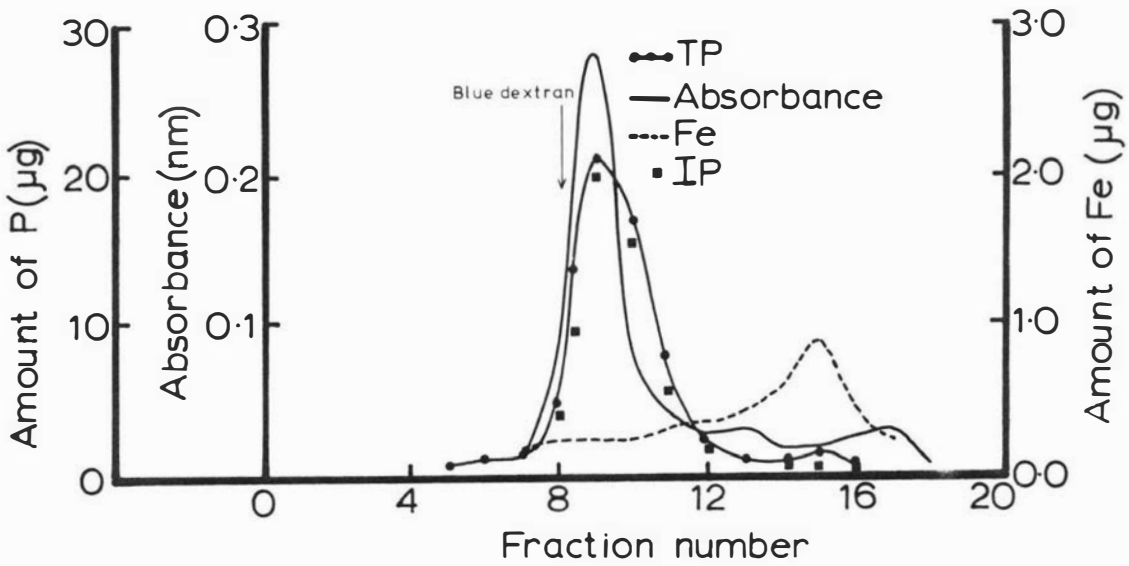

Fig. 9.14 Fractionation of Sephadex G-50 fractions 27 - 32 on Sephadex G-10 using 0.1M NaOH as the eluant (sample size = 0.5ml, column = 15.5 x 1.2cm, and fraction volume = 0.82ml).

The association between high molecular weight organic matter (OM) Fe, and P, obtained in this study, has also been reported by Sinha (1971). This worker found that humic acids extracted from soils by alkali, could only form stable complexes with IP when Fe or Al were also complexed. The presence of OM-Fe-P complexes in soils has been reported by Lévesque (1969), who showed that whereas metal ions were necessary for bridging P and fulvic acid, P may in turn have bridged the fulvic acid-metal units. The results presented in the present study suggest that OM-Fe-P complexes are the major forms of humic-associated TP in the Tokomaru soil. Humic-P complexes, without Fe, may exist in the soil but are possibly less stable during extraction. It is not possible to establish the exact amount of humic-metal-phosphates present in the Tokomaru soil, because such complexes may form in alkaline solutions (Jambu et al., 1972) and during the concentration of OM extracts (Plumb and Lee, 1973). The fact that similar high molecular weight OM-metal-P complexes have been extracted from soils by chelating resins (Levesque and Schnitzer, 1967a), however, indicates that complexes such as those found in alkaline soil extracts do exist in soils. Lévesque and Schnitzer (1967b) suggested that an appreciable portion of soil P exists in the form of such complexes. In addition to IP, small OP molecules may be associated with these complexes because Moyer and Thomas (1970), Veinot and Thomas (1972), and Hong and Yamane (1976) have reported the presence of inositol polyphosphates in humic acids, although they may be limited to associations with the medium molecular weight (1,000 - 50,000) fractions (Moyer and Thomas, 1970).

The essentially complete availability of the 0.1M-NaOH P fraction in Tokomaru soil, during a bioassay with Anabaena (Section 5), suggests that humic-metal-P complexes present in the Tokomaru soil are biologically

available under conditions of the bioassay. The ability of humic-material to promote algal growth has long been known (Pringsheim, 1912), and the growth of nuisance blooms of blue green algae have been associated with the presence of aquatic humus (Shapiro, 1957; Lange, 1970). It has in fact been suggested that the promotion of algal growth is due to the ability of humic acids to chelate trace elements (Prakesh et al., 1973) and therefore increase their availability to algae, or to the removal of toxic substances inhibiting algal growth. de Haan (1972), however, showed that an Arthrobacter sp. isolated from lake water, was able to grow slowly on humus as the only carbon source. The results presented in the present study suggest that the availability of the humic-associated P may also play a part in promoting the growth of bacteria and blue-green algae.

Fractionation of the fulvic material from Tokomaru soil using Sephadex G-25 (Fig. 9.2) indicated that small OP molecules, of approximate molecular weight < 1000, were present in the 0.1M NaOH extract. Moyer and Thomas (1970) have also shown that organic P of similar molecular weight can be extracted from Canadian soils using milder methods of extraction. These workers have also reported the presence of a dark-coloured, organic matter fraction containing very little OP which was eluted last from Sephadex gel, as shown in this study. The fact that in their study, the dark fraction was eluted after the IP peak led them to suggest that adsorption of the organic matter by the gel had occurred and that the peak probably consisted of aromatic material. Steward and Tate, (1969) showed that IP was partially excluded from Sephadex G gels, and this may therefore complicate such interpretations. Elution with 0.1M NaOH as used in the present study, appears to have partially eliminated

adsorption effects and has not apparently increased the exclusion of IP. Similar partial elimination of adsorption effects by alkaline eluants has been demonstrated by Swift and Posner (1972).

Because of the charge exclusion effects between small, ionic organic phosphates and the Sephadex G gels, it is impossible to reliably identify the molecular weight of the OP fractions obtained using Sephadex G-50; an example is iP_6 which was eluted with a Kd of 0.40 when its molecular size suggested that it should have a Kd value of 1. The more complex OP compounds, which have been shown to be non-ionic from their non-adsorption by ion-exchange resins, probably have elution positions related to their molecular size. Only small amounts of inositol polyphosphates appeared to be associated with the OP material excluded from Sephadex G-50 (Fig. 9.7 and 9.10), which presumably has a molecular weight > 30,000. This appeared to constitute only a small amount of the OP in the 0.1M NaOH fulvic extracts from the two soils. The fractions apparently containing medium molecular weight OP compounds, which were partially included in the gel, also contained larger amounts of inositol polyphosphates (Fig. 9.8 and 9.11) than the excluded OP fractions. It was therefore difficult to determine accurately the amounts of complexed OP of medium molecular weight present in the fulvic extract because smaller molecular weight, ionic P compounds were also eluted in the respective Sephadex G-50 fractions. Ion-exchange chromatography of the medium molecular weight, Sephadex G-50 fractions of the Manawatu soil extract (Fig. 9.11), suggests that the majority of OP present in these fractions is in the form of complexed OP, rather than free inositol polyphosphates.

The method of Steward and Tate (1971) appeared to be effective in

the estimation of free inositol polyphosphates in alkaline-soil extracts. The method indicated that small amounts of inositol polyphosphates were present in the Tokomaru extract with slightly higher amounts in the Manawatu extract. These facts were confirmed by the small amounts of inositol polyphosphates identified in these fractions by ion-exchange chromatography. It was evident, however, that the total amount of inositol polyphosphates present in the extracts may be significantly higher than the amount estimated by the method of Steward and Tate (1971), because inositol polyphosphates complexed with low and high molecular weight material were not eluted in the main inositol polyphosphate peak (Kd = 0.4). Similar associations between complex OM and inositol phosphates have been reported by Moyer and Thomas (1970), Osomoto and Wild (1970), and Hong and Yamane (1976).

The nature of the OM-Fe-P associations in the lower molecular weight fulvic fractions has been clarified by their fractionation using Sephadex G-10 (Fig. 9.14). The majority of Fe was partially included in the gel, while the IP and OP were excluded, probably by charge exclusion effects, which increase as the degree of cross-linking in the gel increases (Steward and Tate, 1969). A small amount of OP was associated with the Fe peak (Fig. 9.2 and 9.14), which is consistent with the findings of Plumb and Lee (1973). These workers suggested that for Fe to pass through the gel column, the Fe has to be complexed with organic compounds. Because the elution pattern for the soluble hydroxoferrate ion on Sephadex G gels is not known, the presence of hydroxoferrate in the elution peak cannot be ruled out. In a study of the OM in lake water using Sephadex G-75, Gjessing (1965) found that a large concentration of Fe was eluted slightly ahead of the last OM peak. As shown in Fig. 9.2, this

also occurs in the fractionation of soil fulvic extracts. These results suggest that Fe is not associated with the highly-coloured OM. This is confirmed by the Sephadex G-10 fractionation results (Fig. 9.14) which also suggest that Fe is only associated with a small amount of OM, which is not highly coloured, and with which a small amount of OP is also associated. The reversal of the elution positions of the Fe and OM, when using Sephadex G-10 (Fig. 9.14) instead of Sephadex G-25 (Fig. 9.2), suggests that the OM has a molecular weight < 1000 (below the separation range of Sephadex G-25) and > 700 (above the separation range of Sephadex G-10).

The forms of OP extracted from the Tokomaru and Manawatu soils by 0.1M NaOH can be summarized as follows:

Humic OP (for the Tokomaru soil only) consisted of

OM-Fe-P complexes with molecular weights 10,000 - 70,000.

Fulvic OP constituted the major amount of OP in the extract and consisted of a small amount of OP with a molecular weight > 30,000, with which only small amounts of Fe and inositol polyphosphates were associated; a medium molecular weight complex OP fraction which was associated with Fe and may have contained inositol polyphosphates; a small amount of free inositol polyphosphates; and a low molecular weight complex OP fraction which was associated with high concentrations of Fe and contained inositol polyphosphates.

The bioassay studies in Sections 5 and 6 indicated that for the Tokomaru and Manawatu soils, the majority of the above-mentioned OP forms are available for the growth of Anabaena under the bioassay conditions employed. Other studies which may indicate humic P availability were

discussed previously. There appears to be no published reports concerning the availability of soil fulvic OP to microorganisms. de Haan (1977), however, has shown that fulvic acid isolated from lake water can act as the sole carbon source for an Arthrobacter sp. Presumably, the degradation of the fulvic material by bacteria would also release some of the associated P.

Although the biological availability of fulvic OP has not been studied, several OP compounds, some of which have been shown to be associated with fulvic complexes, can act as P sources for microorganisms. Bacterial utilization of inositol hexaphosphate as both carbon and P sources has been shown by Greaves and Webley (1969) and Greenwood and Lewis (1977). Rodel et al. (1977) have reported the microbial hydrolysis of glycerophosphate and nucleotide phosphates sorbed on lake sediments. Also, Reichardt (1971) has found that several OP compounds can be utilized by blue-green algae as P sources and that the efficiency of use of the OP compounds was increased when algae were previously P starved; surprisingly, the blue-green alga Aphanizomenon sp. was found to grow better with inositol hexaphosphate as a P source, than with dissolved IP. It is generally viewed that whereas soluble OP compounds can act as available P sources for microorganisms, insoluble forms such as Fe and Al inositol hexaphosphates, and forms sorbed by soils (Greaves and Webley, 1969) or lake sediments (Rodel et al., 1977) are not easily hydrolyzed by extracellular enzymes of microorganisms. The partially soluble Ca salt of inositol hexaphosphate, however, can be hydrolyzed considerably by soil yeasts (Greenwood and Lewis, 1977). Although the results of Greaves and Webley (1969) suggest that soil OP may not be expected to show the high degree of availability obtained in this study, the major differences

in experimental procedure and test organisms may account for the contrasting results. The much lower soil:solution ratios used in the present incubations would lead to a greater desorption of IP (Hope and Syers, 1976) and presumably of OP from the soil. In the present study a complete nutrient media, which would stimulate the production of biomass and create more vigorous conditions for OP depletion (Section 8), was added to the incubations of soil material. Such was not the case in the work reported by Greaves and Webley (1969). Also, relatively large inocula of P-starved Anabaena, with a known increased production of hydrolytic enzymes (Section 6.8; Healey, 1973) were added to the soil materials (OP source) in this study, whereas in the study of Greaves and Webley (1969) the OP was added to soil systems that presumably had low microorganism populations due to natural nutrient limitations. More importantly, Anabaena species are known to produce hydroxamate chelating agents capable of chelating Fe (Murphy and Lean, 1976; Section 8). Presumably, because of the close association between Fe and OP noted in the fulvic extracts from these soils, the chelation of Fe would result in OP being simultaneously released to solution, where it is more susceptible to hydrolysis.

The above observations may explain why under conditions favouring the depletion of soil OP and vigorous algal growth, the blue-greeen alga, Anabaena, can use the 0.1M NaOH-OP fraction from the Tokomaru and Manawatu soil materials. This ability may be aided considerably by increased bacterial decomposition of the fulvic material. Cometabolism, which is the microbial degradation of a resistant substance in the presence of readily-degradable substrate, of fulvic acids has been shown by de Haan (1974). The extracellular nitrogenous compounds produced by blue-green algae have also been found to stimulate the growth of some bacteria

(Fogg, 1962; Watt, 1969; Whitton, 1973), and may act as the readily-degradable substrate.

The data obtained in this study indicate that OP in fine particulate material can be an important source of biologically-available P. Consequently, in any study of the biological availability of particulate phase P in waters, close attention should be paid to the amount and nature of particulate-phase OP.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The work presented in this thesis may be summarized as follows:

1. A review of the literature indicated that very few attempts have been made to chemically characterize the amounts and forms of biologically-available particulate P in soils and sediments. Such studies have been concerned only with lake sediments and there appear to be no published data relating to the chemical characterization of biologically-available P forms in stream sediment and in surface-runoff materials. Furthermore, previous studies have not evaluated the importance of particulate organic P (OP) as a biologically-available P form.
2. Procedures for the bioassay of particulate P using Anabaena subcylindrica were developed and evaluated. The measurement of chlorophyll concentration and whole cell alkaline phosphatase activity were established as reliable indices of biomass and algal P status, respectively. Algal P content (as determined by extraction with 0.1M NaOH) decreased as external P availability decreased and, therefore, was directly related to biomass only when P availability was constant. By culturing Anabaena in systems containing P sorbed on Fe gel it was possible to control P availability to the algae, and to produce Anabaena of similar P status to Anabaena grown in soil and sediment systems.
3. A combined bioassay-P fractionation procedure was developed and preliminary evaluation studies with Tokomaru soil (< 30μm) indicated that a considerable proportion of NaOH-soil-P was available for

algal growth. Anabaena produced in Fe gel systems and having similar P status to Anabaena in the soil systems, was chemically fractionated and provided estimates of the algal P contribution to extractable soil-algal P. In this way the depletion of soil P was calculated. The amount of biologically-available P was less than the amount of 1M NaOH-soil-P. Subsequent modification and evaluation of the P fractionation procedure indicated that for the Tokomaru and Okaihau soils (< 30μm), 0.1M NaOH-extracted almost exclusively, the particulate P which was biologically available, of which the larger part was particulate OP. The soil P forms extracted by 1M NaOH CDB, and HCl subsequent to a 0.1M NaOH extraction were not available for algal growth.

4. High biological availability was exhibited by the 0.1M NaOH-OP fraction for all materials studied, under conditions favouring the depletion of particulate P. For soils of low P status with a high P sorption capacity, 0.1M NaOH-soil-IP tended to overestimate biologically-available IP. The amount of 0.1M NaOH-soil-P in allophanic material considerably overestimated the amount of biologically-available P because the P absorbed by allophane was extracted by 0.1M NaOH. The data suggested that IP and OP adsorbed by soil components had high biological availability, whereas IP absorbed by these materials and present in apatite exhibited very low or no biological availability. In non-shaken, minimally-disturbed bioassays, Anabaena was still capable of depleting large amounts of 0.1M NaOH-soil-P. The extraction of a water sample containing particulates with 0.1M NaOH was proposed as a method for estimating the maximum amount of biologically-available P present in the sample.

5. Particulate OP was found to be a source of biologically-available P. The increased utilization of this form in the presence of increased algal biomass and increased alkaline phosphatase activity suggested that extracellular enzymes play an important role in its depletion. In several materials, 0.1M NaOH-OP was depleted to a greater extent than 0.1M NaOH-IP.

6. The amounts of biologically-available P, estimated by the developed procedure, in soil materials (< 30μm) were much smaller than the amounts of soil P extracted by persulphate digestion. The amounts of particulate IP exchangeable with ^{32}P during 30 min appeared to provide estimates of the amounts of soil IP that could be rapidly assimilated by algae. As with exchangeable P values determined for longer periods of exchange, these amounts were considerably smaller than the amount of soil IP shown to be biologically available.

7. The simple desorption of P accounted for only a small amount of the particulate P used for algal growth. With a highly fertilized soil, containing a greater amount of readily-desorbable P, this mechanism appeared to be more important. Algal-soil contact was required for the maximum amount of particulate P to become available for algal growth.

8. Increases in solution P concentration enhanced the ability of Anabaena to deplete particulate P. Decreases in solution P concentration produced the reverse effect. Initial availability of particulate P was found to be important in governing the extent of subsequent algal growth and particulate P depletion by Anabaena. This observation has direct relevance to the effect of the initial concentration of biologically-available P on algal growth in natural waters.

9. Biologically-available OP in two soils was extracted by 0.1M NaOH and was characterized by separation into humic and fulvic extracts and their subsequent fractionation on agar gel and Sephadex gel columns, respectively. Humic OP consisted mainly of high molecular weight organic matter-Fe-P complexes. The fulvic extracts constituted the major part of the 0.1M NaOH-OP and contained both high and low molecular weight organic matter-Fe-P complexes. Inositol polyphosphates, both free and complexed, were also identified in the fulvic extracts by ion-exchange chromatography.

10. The data obtained in this study demonstrate that soil and sediment material can act as a major source of IP for the growth of algae under conditions favouring the optimum depletion of P. In addition, it has been demonstrated that particulate-phase OP can be an equally or more important source of P for algal growth; an observation which does not appear to have been reported previously.

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APPENDIX

APPENDIX

The effect of growth media ASM-1, P-, N- (Gorham et al., 1964) on solution P concentration

It is conceivable that the presence of certain anions and the chelating agent EDTA in the ASM-1, P-, N- growth media may cause the desorption of P from the soil and, therefore, increase P availability during algal growth to an artifically high level. To investigate this possibility soil:solution P relationships were compared in distilled water and ASM-1 media. The <30-μm fraction (1g) of Tokomaru silt loam (0 - 5cm) was shaken for 3 days in flasks containing 1 litre of distilled water or 1 litre of ASM-1-media. After shaking, aliquots of suspension from each flask were chemically fractionated using procedures described in Section 3. The results of the P fractionation are shown in Table I.

The difference between the P fractions recovered from Tokomaru silt loam suspended in water and in the growth medium was very small. The same finding applied to the concentration of P sustained in solution (designated Solution in Table I) after 3 days by both systems; this very small difference in Solution concentration would be unimportant for algal growth.

It was concluded that the ASM-1 growth media had no direct, measureable effect on the avialability of particulate P. Estimates made of biologically-available P would therefore be the result of algal or bacterial action, rather than any chelating effect of the growth media.

APPENDIX

Table I Effect of ASM-1, N-, P- nutrient media on solution P concentration and on the amounts of extractable-soil P from Tokomaru soil (< 30-μm)

		Amount of P extracted (μg P g^{-1}) by	
Extract		ASM-1, P-, N-	Distilled water
Solution	IP	5.3	3.8
	TP	13.8	14.0
0.5M NH_4Cl	IP	4.8	4.8
	TP	7.4	6.4
1M NaOH	IP	78	77
	TP	320	312
CDB	IP	25	28
1M HCl	IP	15	13

APPENDIX

Table II Chlorophyll concentration, alkaline phosphatase activity (APA), and extractable-algal P during the growth of Anabaena in Fe gel systems

Fe gel systems	Time (days) 0	1	7	10
	Chlorophyll concentration ($\mu g\ ml^{-1}$)			
P-	0.25	0.25	0.35	0.36
A	"	0.27	0.35	0.47
B	"	0.27	0.67	0.92
C	"	0.28	0.99	1.37
D	"	0.28	1.64	2.28
E	"	0.26	1.78	2.50
F	"	0.25	1.92	2.73
	APA (μ moles pNP hr^{-1} μg^{-1}chl			
P-	0.12	0.16	0.71	0.78
A	"	0.16	0.64	0.44
B	"	0.15	0.53	0.41
C	"	0.14	0.43	0.38
D	"	0.12	0.23	0.32
E	"	0.09	0.12	0.22
F	"	0.06	0.05	0.12
	0.1M NaOH-algal-IP ($\mu g\ l^{-1}$)			
P-	14	18	22	18
A	"	14	16	18
B	"	16	19	25
C	15	17	20	31
D	"	20	23	41
E	"	23	36	57
F	"	27	41	68
	0.1M NaOH-algal-TP ($\mu g\ l^{-1}$)			
P-	37	47	55	53
A	"	31	58	56
B	"	38	77	112
C	"	38	123	166
D	36	49	135	250
E	45	66	181	273
F	47	84	270	293
	1M NaOH-algal-TP ($\mu g\ l^{-1}$)			
P-	2	2	16	-
A	2	-	36	-
B	5	7	27	-
C	5	7	39	-
D	4	7	53	-
E	5	7	76	-
F	-	23	116	-

APPENDIX

Table III Chlorophyll concentration, alkaline phosphatase activity (APA), and extractable-algal-P during the growth of Anabaena in Fe gel systems

Fe gel system	Time (days) 1	5	8	12	19	22	26
	Chlorophyll concentration ($\mu g\ ml^{-1}$)						
A, P-	0.40	1.03	1.12	1.25	1.13	-	-
B	0.38	0.86	1.28	1.46	1.60	1.63	-
C	0.41	1.25	2.15	2.89	3.59	4.65	4.30
D	0.44	1.44	2.50	3.73	4.22	6.13	6.73
	APA (μ moles pNP $hr^{-1}\ \mu g^{-1}$ chl)						
A, P-	0.04	0.07	0.22	0.37	1.37	-	-
B	0.04	0.06	0.17	0.343	1.06	-	-
C	0.04	0.02	0.05	0.06	0.23	0.26	-
D	0.03	0.01	0.01	0.02	0.062	0.10	-
	0.1M NaOH-algal-IP ($\mu g\ l^{-1}$)						
A, P-	87	94	72	75	63	-	-
B	67	41	48	65	73	-	-
C	67	51	56	87	100	122	122
D	85	103	63	112	167	174	223
	0.1M NaOH-algal-TP ($\mu g\ l^{-1}$)						
A, P-	200	241	218	243	245	-	-
B	216	245	254	326	344	-	-
C	221	315	343	445	528	557	584
D	300	463	610	800	854	889	920

APPENDIX

Table IV Chlorophyll concentration, alkaline phosphatase activity (APA), and extractable-algal-P during the growth of *Anabaena* in Fe gel systems

Fe gel system	Time (days) 0	1	3	7	10	14
	Chlorophyll concentration ($\mu g\ ml^{-1}$)					
P-	0.26	0.37	0.37	0.39	0.33	-
A	"	"	0.38	0.63	0.68	-
B	"	"	0.40	0.91	0.98	1.11
C	"	0.35	0.43	1.46	1.51	0.50
D	"	0.32	0.56	1.99	2.18	1.41
	APA (μ moles pNP hr^{-1} μg^{-1} chl)					
P-	0.015	0.16	0.43	0.79	1.37	2.04
A	"	0.12	0.38	0.70	1.02	1.66
B	"	0.08	0.33	0.61	0.68	1.29
C	"	0.02	0.06	0.24	0.46	0.95
D	"	0.02	0.09	0.19	0.28	0.73
	0.1M NaOH-algal-IP ($\mu g\ l^{-1}$)					
P-		11	9	13	-	13
A		12	12	22	-	25
B		13	15	32	-	48
C		22	22	83	-	96
D		37	52	115	-	159
	0.1M NaOH-algal-TP ($\mu g\ l^{-1}$)					
P-		30	30	28	-	27
A		40	49	65	-	103
B		50	78	135	-	174
C		50	128	221	-	375
D		99	167	272	-	435

APPENDIX

Table V Chlorophyll concentration, alkaline phosphatase activity (APA), and extractable-algal-P during the growth of Anabaena in Fe gel systems

Fe gel system	Time (days) 1	7	10	15	17	27
	Chlorophyll concentration ($\mu g\ ml^{-1}$)					
P-	0.275	0.220	0.152	0.779	0.109	-
A	0.275	0.264	0.291	-	0.359	0.436
B		0.283	0.305	-	0.373	0.441
C		0.305	0.319	-	0.387	0.444
D		0.319	0.324	-	0.387	0.458
E		0.332	0.332	-	0.389	0.471
F		0.297	0.332	-	0.359	0.471
G		0.340	0.389	-	0.422	0.526
	APA (μ moles pNP hr^{-1} μg^{-1}chl)					
P-	0.038	0.836	1.47	1.78	2.57	3.00
A	0.022	0.338	0.427	0.680	0.704	0.704
B	0.021	0.315	0.354	0.480	0.521	0.564
C	0.020	0.291	0.282	0.281	0.336	0.428
D		0.244	0.164	0.163	0.207	0.389
E		0.197	0.046	0.041	0.079	0.351
F		0.141	0.020	0.014	0.062	0.075
G		0.045	0.004	0.022	0.034	-
	0.1M NaOH-algal-TP ($\mu g\ l^{-1}$)					
P-	16	12	-	-	34	-
A	21	35	-	-	94	-
B	23	47	-	-	101	119
C	26	59	-	-	138	158
D	40	85	-	-	198	236
E	54	112	-	-	258	314
F	57	136	-	-	315	412
G	93	193	-	-	524	652
	0.1M NaOH-algal-IP ($\mu\ g\ l^{-1}$)					
P-	6	4	-	-	18	-
A	7	12	-	-	36	-
B	7	14	-	-	46	54
C	8	16	-	-	57	65
D	9	25	-	-	69	81
E	10	35	-	-	81	98
F	11	41	-	-	91	119
G	20	52	-	-	113	141