A CHARACTERIZATION OF MOBILE FORMS OF PHOSPHORUS IN A CALCAREOUS SOIL

by

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I hereby recommend that this dissertation prepared under my direction by Mostafa Hassan Mohamed Hilal

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be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

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ABSTRACT

An investigation was undertaken to characterize the phosphorus compounds moving downward in a calcareous soil. Duplicate columns, filled with calcareous soil material taken from the B horizon of Mohave sandy loam, were treated with sucrose + NH_4NO_3, bean residue and no treatment. Phosphate compounds moving with the soil solutions of these columns were studied with respect to their physical as well as their chemical nature. Millipore filtration and anion exchange chromatography were the principal means for physical fractionation and chemical partition respectively.

Phosphorus movement in the untreated soil was generally low with more than 50 percent of the mobile phosphorus being organic. Application of organic matter increased the movement of organic phosphorus 8 to 11 fold and caused little change in the movement of inorganic phosphorus. Millipore filtration showed that more than 70 percent of the mobile organic phosphorus was associated with particles larger in size than 0.45μ and that 20 percent of it was associated with particles of 0.45μ to 10 mμ in size. Only 10 percent or less of total organic phosphorus moving with the soil solutions was actually water soluble. Microscopic examination of soil solution showed that microbial cells were the predominant particles in the soil solutions.

The major part of mobile organic phosphorus was most probably sequestered within these microbial cells. It is probably due to the particulate nature that the mobile organic phosphates are not available to plants.

Eighty to 85 percent of total phosphorus moving with the soil solution was KOH-soluble. Fractionation of the KOH-soluble phosphorus through AG 1-X4 resin showed that 39 to 44 percent of total KOH-soluble organic phosphorus was associated with RNA and RNA-related compounds. Ultra violet absorption spectra substantiated such finding. Phosphate esters, resembling glucose phosphates and glycerophosphates in behavior were present in small amounts in the KOH extracts of the soil solution. The acid soluble fraction, which probably contained a considerable amount of phytate phosphorus and related compounds, accounted for a large portion of phosphorus movement. Phytin moving with soil solution of sucrose treated columns was probably from microbial origin since the plant materials were present in insignificant amounts if not absent. Small amounts of phospholipids were extracted from the colloidal material moving downward with the soil solution of a sucrose treated column.

It was concluded that microbial cells and cellular debris were the principal phosphorus carrying particles in the soil solution and whatever the phosphorus they contain will account for a large portion of mobile phosphorus.

INTRODUCTION

The mobility of phosphorus compounds with the soil solution is important in plant nutrition and soil formation; however, little work has been devoted to the study of organic phosphorus movement in the soil. Most of the research on movement has been concerned with the diffusion and mobility of inorganic orthophosphate (34) with little attention having been given to the distinction between organic and inorganic phosphorus in the soil solution (48).

Several studies in the humid regions, which are well supplied with organic matter, have indicated the important role of organic phosphorus in the overall phosphorus status and fertility of the soil. The predominance of organic phosphates in the displaced soil solution and in the soil-water-extract was demonstrated (48). In the arid and the semi-arid regions where calcareous soils predominate, organic phosphorus also has been shown to be equally important (30). Recently it was found that the treatments which increased phosphorus movement in a calcareous soil did so by increasing the movement of organic phosphorus (34).

Once the importance of organic phosphate in the soil solution was demonstrated, it became of interest to determine its availability to plants as compared to that of inorganic phosphate. A marked increase

in soil phosphorus availability has been obtained upon adding organic matter to some soils (25); however, the addition of energy source to a soil of initial low phosphate availability has resulted in a marked decrease in plant yield (18). Whether the addition of organic material would result in initial mineralization or initial immobilization of soil phosphorus was found to depend on C/P ratio of the added organic material. Organic material having C/P ratio larger than 200 was shown to result in initial immobilization of soil phosphorus (32).

A large part of the phosphorus of the plant tissue has been shown to be organically combined as nucleic acids, phytin and lecithin (39). Since the residues of higher plants and microorganisms are the principal source of organic phosphorus in soils, it is to be expected that these compounds and their intermediate decomposition products constitute the bulk of the organic phosphorus found in soils. Several investigations have shown the presence in soils of nucleic acid, nucleotides and phytin in relatively large amounts and lecithin in small quantities (50).

Absorption experiments indicated that organic phosphorus present in soil solutions or soil water extracts was unavailable to plants (48). However, plants could absorb phosphates from added organic phosphates such as phytin, lecithin, nucleic acids, nucleotides and glycerophosphates at much the same rate as from K_2HPO_4 (50).

Results such as these have led to a dilemma. What are the organic phosphorus compounds that move with the soil solution? Are these compounds different from those tested in the absorption experiments, namely phytin, lecithin, nucleic acids, nucleotides and glycerophosphates? If they are the same, what then is the cause for the difference in their relative availability to plants?

If these are the major constituents of organic phosphorus in the soil, one can hypothesize that these compounds comprise at least a part of the organic phosphorus present in soil solution. The possible reason for their unavailability to plants when present in soil solution may be that they are not actually water soluble but rather particulate in nature.

This hypothesis was tested by studying phosphate movement in a calcareous Mohave soil with specific attention given to the physical disposition and chemical partition of organic and inorganic phosphorus compounds migrating with the soil solution.

The chemical and physical nature of mobile soil phosphorus was studied using sucrose and a plant residue both of which have been known to increase phosphorus movement. Physical fractionation of the soil solution was accomplished by using Millipore filters of different pore sizes. Some chemical components isolated by base extraction combined with anion exchange chromatography were characterized.

LITERATURE REVIEW

The Downward Movement of Phosphorus in Soils

The liquid phase of the soil, or soil solution is the most mobile, variable and active soil component. The products of soil formation and weathering move in the soil solution within the soil profile in the form of ions, molecules, colloids and cell materials. Decomposition of organic matter, disintegration of mineral particles and the synthesis of organic mineral compounds are some of the transformations that take place in the soil solution.

The uptake of nutrients and water by plants is associated with the soil solution which is in contact with both the solid phase of the soil and the plant roots. "Soil solution by sustaining life and the existence of plant tops is probably the most important element of the mechanism of the biosphere on land" (38).

One way of obtaining the soil solution is by displacement. Experimental evidence has shown that the displacement method gives a solution identical with or at least very similar to the true soil solution (13).

Since the development of the displacement method of obtaining the soil solution (13), several investigators have made studies on the phosphorus content of the displaced solution (30, 31, 34, 35, 44, 46,

48). However, of the amount of work directed towards the study of phosphorus behavior in soils, relatively little has been devoted to phosphorus movement in the soil solution, especially in calcareous soils. Much of the work has been concerned with the diffusion of inorganic phosphate and its replenishment from native soil phosphorus or from the phosphorus fertilizer (17, 19, 45).

As early as 1927 Pierre and Parker (48) studied the concentration of organic and inorganic phosphorus in the displaced soil solution and in the soil extract of a number of representative soils. They demonstrated the importance of organic phosphorus when they found that the concentration of organic phosphorus in the soil solution averaged 0.47 ppm as $PO_4^{\equiv}$. Soil solutions of almost all the soils studied contained very low concentrations of inorganic phosphorus. Organic phosphorus, on the other hand, represented 84 percent of total phosphorus present in the soil solution.

Several other studies in the humid regions (2, 10, 47) emphasized the important role of organic phosphorus in the overall phosphorus status and fertility of the soil.

In the arid and semi-arid regions where low organic matter calcareous soils predominate, the organic phosphorus was also found to be important. Fuller and McGeorge (30) found that about one-third of the total phosphorus in several calcareous soils was in the organic form. They added that considerable amounts of organic phosphorus

appeared in water and carbonic acid extracts of the soils. This finding was rather surprising in view of the low organic matter content of these desert soils. Many cultivated Arizona soils were shown to be completely lacking in water-soluble and "CO_2-soluble" inorganic phosphorus. However, small quantities of organic phosphorus were nearly always present in the extracts (31). Carbon dioxide-soluble organic phosphorus represented values up to 60 percent of the total CO_2-extractable phosphorus.

Spencer and Stewart (55) examined the penetration of several added organic and inorganic phosphorus compounds in a calcareous soil. They found that more than 90 percent of the added inorganic phosphorus was fixed as compared with only 5 to 20 percent of the added organic form.

Based on this unique experiment, Hannapel et al. (34) hypothesized that in calcareous soils considerable phosphorus is transported in various organic forms by means of saturated flow of water. They examined the influence of plant residues as well as sucrose, a microbial energy source, on both magnitude and form of phosphorus moving in the soil solution of a calcareous Arizona soil. All organic matter treatments accelerated the movement of total phosphorus. Such increase in movement of total phosphorus was mainly due to the increase in movement of organic phosphorus. Addition of inorganic phosphorus failed to cause any increase in phosphorus movement in the absence of added organic material.

Hannapel et al. (34) thought that the increased movement of organic phosphorus was due to the movement of microbial tissues, cells and debris. Indeed, Chouchack (18) and Kaila (37) have suggested that much of the "soluble" organic phosphorus is present within microbial cells. Hannapel et al. (34) observed an increased turbidity in those displacements of soil solutions which contained large quantitites of organic phosphorus. Furthermore, the Millipore filtration, through filters of 0.45 μ pore size, indicated that a large portion of the organic phosphorus was associated with microbial cells and cellular debris. The addition of a microbial energy source greatly accelerated the movement of phosphorus with more than 95 percent of the moving phosphorus being organic.

Treatments with formaldehyde suppressed the activity of the microbial population and resulted in a reduction of phosphorus movement. Assuming that the 0.1 percentage formaldehyde treatment had little effect on the solubility of phosphorus compounds in the soil, it was concluded that this reduction in phosphorus movement must have been due to adverse effects of formaldehyde on microorganisms (35).

This transport of microorganisms in soil solution is supported by the findings of Hepple (36) who has shown down movement of small fungal spores, ranging in diameter from 2 to 3 μ, in the sandy A_1 and A_2 horizons of a podzol. Morowitze (43) reported the existence of small living cells that have diameters of only 0.125 to 0.150 μ.

The importance of organic phosphate in the soil solution is beyond doubt. More attention should be directed towards the study of the nature of mobile organic phosphates. The role of soil microflora in transporting phosphorus compounds should not be neglected.

Availability of Phosphorus Forms to Plants

Once the importance of organic phosphate in the soil solution is demonstrated, one would raise the questions which were raised before by Pierre and Parker (48): Can the plant absorb organic phosphorus? Is the organic phosphate as available to plants as the inorganic phosphate?

Pierre and Parker (48) stated that it has been assumed in the past that all of the water soluble phosphate is inorganic or at least the assumption has been made that all water soluble phosphorus is available to plants. They doubted the validity of such an assumption due to the fact that their data showed the predominance of organic phosphate in the displaced soil solution.

Chouchak (18) showed that the addition of microbial energy source to a soil of low phosphorus availability resulted in a marked decrease in yield of millet, a decrease which could be prevented by adding phosphate fertilizer. Dalton (25), on the other hand, obtained evidence showing a marked increase in soil phosphorus availability when organic matter was added to the soil.

To solve such a conflict, Fuller et al. (32) found that whether the addition of organic material would result in an initial immobilisation or an initial mineralization of soil phosphate would depend upon the carbon to phosphorus ratio of the organic material added. Initial immobilization was shown to occur when the C/P ratio was above 200. The addition of sucrose alone to a soil would be expected to produce initial immobilization. Bogan et al. (11) employed algae as a means of biologically removing phosphate from sewage. They also demonstrated the influence of energy source on immobilization or "biological absorption" of phosphorus when they found that the presence of adequate amount of light resulted in a more rapid biological extraction of phosphate. The availability of phosphorus forms and the influence of microorganisms were also discussed by other workers (12, 57, 58).

Treating a soil with a carbon source such as sucrose was found to cause immobilization of phosphorus for plant use and the same treatment was shown to increase phosphorus movement. The two phenomena may seem, in the first instance, somewhat anomalous. But, Hannapel et al. (34) stated that if the increased movement of phosphorus was associated with the migration of microorganisms within the soil solution, quite obviously the immobilisation in and movement of microbial cells would resolve the apparent anomaly.

Along with the availability problem, Pierre and Parker (48) showed that plant roots could not utilise the organic phosphorus forms

present in the soil solution or soil extract. Experiments with corn showed that growth was almost proportional to the inorganic phosphorus present in soil extracts. Parker (46) explained the inability of the displaced soil solution to support plant growth on the basis that plant roots exert a solvent action on the particles and thus bring more phosphorus into solution, producing a higher phosphate concentration at the absorbing surfaces.

El-Bagoury (29) did not find any correlation between the organic phosphorus content of the soil and its availability to plant in some Canadian soils.

Eid et al. (28) indicated that at soil temperature of 20°C. the availability of soil phosphorus to plants was determined by only inorganic phosphorus. Soil organic phosphorus had no appreciable effect. At soil temperature of 35°C., on the other hand, both inorganic phosphorus and organic phosphorus were significantly related to the amount of plant-available phosphorus. The effective organic fraction was the organic phosphorus soluble in hot one percent K_2CO_3 and hydrolyzed by hypobromite. The K_2CO_3-soluble organic phosphorus fraction that was not hydrolyzed by hypobromite and the K_2CO_3-insoluble organic phosphorus fraction were not related to the plant-available phosphorus. Eid et al. (28) explained the effect of temperature on the basis of rapid mineralization of a certain fraction of organic phosphorus.

Rogers et al. (50) found that organic phosphorus present in the soil solution or in a soil water extract was not available to corn while that present in NH_4OH extract of the same soil was readily available. On the other hand, they indicated that plants can absorb phytin and lecithin directly from nutrient solutions. Nucleic acids, nucleotides and calcium glycerophosphate decomposed when placed in contact with corn or tomato roots yielding inorganic phosphorus. In other words, plants can absorb phosphorus from added organic phosphates such as phytin, lecithin, nucleic acids, nucleotides and glycerophosphates when in true solution at much the same rate as they do from K_2HPO_4.

These results lead to the following problem: What are the organic phosphorus compounds that move with the soil solution? Are these mobile compounds different than those tested by Rogers et al. (50)? If they are the same, why is it that they differ in their availability to plant roots?

Before attempting a study of the nature of organic phosphorus moving with the soil solution, it is desirable to review the work done on the nature of soil organic phosphorus.

The Chemical Nature of Soil Organic Phosphorus

Soil organic phosphorus is generally considered to be derived from plants, animals or microorganisms either by secretion and

excretion from living cells or from decomposing cellular material. The principal organic phosphorus compounds to be expected from such sources are phospholipids, nucleic acids and inositol phosphates with lesser amounts of phosphoprotein and highly labile compounds such as sugar phosphates, coenzymes and phosphogens. Indeed, Bartholomew and Goring (8) were able to separate phosphorus bearing compounds present in microorganisms into four general fractions which were: the acid soluble, the phospholipid, the nucleic acids and the phosphoprotein fractions. The acid soluble fraction contained, soluble inorganic phosphorus, most of the phosphorus carrying products and agents of intermediate metabolism and phytin if present in the microbial cells. The relative proportion of the phosphorus occurring in each fraction varied markedly among and to a certain extent within the plant, animal and microorganism. They found that the major portion of the organic phosphorus was either the acid soluble or the RNA fraction. The addition of one percent bentonite to the fresh cells markedly altered the distribution of phosphorus among the several fractions. The percentage of RNA-phosphorus was greatly increased while that of the acid soluble phosphorus was markedly decreased due to the adsorptive properties of the clay.

Bartlett (9) was able to isolate inorganic phosphate, sugar phosphates and derivatives, and nucleotide phosphates from red blood cells using anion exchange resins and columns of activated carbon.

Smith and Clark (53) reported that phytin, the mixed calcium-magnesium salt of inositol hexaphosphoric acid accounts for most of the organic phosphorus of seeds. It occurs in smaller proportion in the vegetative parts of the plant. Substantial quantities of this compound therefore normally reach the soil.

The study of soil organic phosphorus depends to a large extent on the methods of its extraction and fractionation. Suitable methods are available for the estimation of total soil organic phosphorus (42, 51, 52). Wells and Saunders (60) indicated that organic phosphorus in New Zealand top soils represents 54 percent of the total soil phosphorus. Cosgrove (21) reported that the organic phosphorus in soils varies from very small values up to 80 percent of the total phosphorus. Bower (12) indicated the possibility of splitting the bulk of soil organic phosphorus into two fractions when treated with Ca^{++} at neutral pH. He considered the Ca insoluble fraction to be inositol phosphates and the Ca soluble fraction was considered to behave like nucleic acids. However, attempts at isolating and quantitative measurement of individual compounds have met with very little success (10).

Estimates have been made for RNA (1), DNA (4), and lipid-P (33), but the total amount of all these compounds did not exceed 5 percent of the total soil organic phosphorus.

Inositol phosphates predominantly myoinositol hexaphosphate have been identified by several workers (3, 14, 20, 21, 27, 53, 61, 62).

The values reported were mostly in range of 12-30 percent of total soil organic phosphorus. Knowledge of the remaining 60-80 percent of the soil organic phosphorus is lacking (7).

Martin (39) stated three possibilities as explanations for this disparity: (a) the occurrence in soil of polymeric phosphate containing compounds such as "teichoic acids" from bacterial cell walls or phosphorelated polysaccarides, (b) the occurrence in soil of stable complexes of inorganic phosphates with soil organic matter which analyze as organic phosphorus, and (c) the occurrence of complexes of soil organic matter and simple organic phosphorus esters which would modify the properties of the esters. Anderson and Hance (6) found that part of the inositol hexaphosphate present in alkaline extract of soil was bound up in a complex containing carbohydrate and proteins. Such finding is in support of (c). Cosgrove (21) also found that the inositol phosphate preparations from several Australian soils were suitable for separation by ion exchange chromatography only following treatments with alkaline hypobromite and hydrolysis with 5N HCl.

Phytin, the major identified fraction, was usually isolated by precipitation as a ferric salt at low pH after the oxidation of the soil organic matter with hypobromite. Such a precipitate was examined by column chromatography. Stepwise elution with HCl of increasing strength was adopted by Smith and Clark (53, 54) to separate the material into a number of fractions among which were inositol

pentaphosphates and hexaphosphates. Caldwell and Black (14, 15, 16) simplified this method and used it for quantitative determination on a wide range of soils. They were able to isolate inositol hexaphosphate and an "isomer of inositol hexaphosphate" from the crude ferric phytate.

Anderson (3) used paper chromatography to separate phytin from other organic phosphates in soil. He estimated phytin as 27 to 38 percent of the soil organic matter.

Gradient elution chromatography has been used by Cosgrove (21, 22) to separate the constituents of phytin fraction of soil organic matter. In addition to the commonly occurring myoinositol hexaphosphate, the presence of the corresponding derivatives of Dl-inositol and scyllo-inositol was demonstrated. Cosgrove (23) was also able to isolate another isomer which was neoinositol hexaphosphate. By using a gas-liquid chromatography, he estimated neoinositol hexaphosphate to be 1 percent of total phytin. Dl- and scyllo-inositol phosphates were also minor components in total soil phytate.

Thomas and Lynch (56) separated soil organic phosphorus into three fractions which contained unidentified phosphorus compounds, myoinositol hexaphosphate and a supposed isomer of the latter. The ratio of these two isomers was considerably different in the different soil horizons. In the A horizon the myoinositol hexaphosphate averaged 43 percent of the total inositol phosphates while in the B horizon it

averaged 62 percent. The low content of the supposed isomer in the B horizon might have been due to the lack of suitable conditions for the growth of the microorganisms responsible for synthesis of the isomer. Both of the inositol phosphates accounted for only 2.8 percent of total organic phosphorus in the B horizon while the same compounds accounted for 11.8 percent of the total organic phosphorus in the A horizon.

The source of inositol hexaphosphates, especially that of isomers other than myoinositol phosphate, is not competely known. Caldwell and Black (15) considered the soil microorganisms as a source of a large proportion of soil phytate, although the synthesis of inositol hexaphosphate by microorganisms in pure cultures has not been demonstrated. They found myo- and scyllo-inositol hexaphosphates in extracts taken from a mixed clay-sand culture which was treated with sucrose and inorganic nutrients, inoculated with soil microorganisms, and incubated for several months at 30°C.

Cosgrove (24) could not detect phytate phosphorus in extracts of soil fungi and yeast cells but he successfully extracted phytin from acorns. Acorn phytin was shown to consist only of myoinositol hexaphosphate although free scyllo inositol was present in the tissues. Small amounts of phytate phosphorus were isolated from sand-clay cultures after a period of incubation, but only myoinositol appeared to be present. If Dl- and scyllo inositol phosphates were formed, they might have been present in quantities below the lower limit of detection.

Acidified cultures (pH value of 4-5) were shown to accumulate phytate phosphorus whereas the less acid cultures did not (24). This was in line with the observation that the acidic alpine humus soils accumulate phytate phosphorus.

Martin (39) criticised the method adopted by Cosgrove (21) for isolating the phytin fraction, in that it involves oxidation by alkaline hypobromite which would result in the destruction of soil organic phosphorus compounds other than inositol hexaphosphate. He also reported the failure of the fractional precipitation method in which phytin is isolated as a ferric salt, which is the method adopted by Caldwell and Black (14). Anderson (5) showed that the inositol phosphates form a range of complexes with widely different solubilities over a range of P/Fe^{+++} ratios; the solubility increasing with the increasing concentrations of Fe^{+++}. This complex formation may be the reason for incomplete precipitation of added inositol phosphates at pH 1.8 and the failure of Caldwell and Black's procedure for estimating phytin.

Martin (39) developed a method which would extract the organic phosphorus as quantitatively as possible with minimum degradation and then separate the organic phosphorus into distinct fractions. The soil organic phosphorus was extracted with 0.3M KOH solution following an acid pretreatment. A fractionation method through anion exchange resin columns was then employed to separate the 0.3M KOH extract into three fractions: (I) humic associated phosphorus which was eluted

with 0.3M KOH solution, (II) inorganic phosphorus which was eluted with 0.85M KOH and (III) the bulk of the acid soluble organic phosphorus which was eluted with 2M KOH.

The behavior of known organic phosphorus compounds added to the 0.3M KOH extract was determined using the resin fractionation method (40). With resin having 3-5 percent cross linking, inositol phosphates, RNA nucleotides and glycerophosphates were quantitatively retained by the resin and could be recovered in good yield, each in a different fraction.

The glycerophosphate was recovered quantitatively in fraction (II) together with the inorganic phosphorus. Presumably other simple monophosphate esters would also elute in this fraction. The amount of organic phosphorus in fraction (II) was small accounting for only 5-10 percent of the total organic phosphorus in Egmont soil (40).

Phytate phosphorus was recovered in good yield in fraction (III). Rechromatography of fraction (III) using the gradient elution method of Cosgrove (21) provided good evidence for the occurrence of inositol phosphates in the Egmont soil. The amount corresponded to 20 percent of the total organic phosphorus. Martin (40) observed a rapid formation of a phosphate complex upon the addition of inositol hexaphosphate to a soil base extract. This finding would throw considerable doubt on the estimates of inositol hexaphosphate content in soil when ion exchange chromatography is employed in the determination.

None of the added RNA-phosphorus was recovered using the standard elution with KOH solutions. However, it was quantitatively recovered on elution with 10 percent HCl. RNA-phosphorus was not present in the non-amended soil extracts. Lack of absorption in the UV spectrum from 260 to 300 mμ further indicated the absence of RNA in the soil extracts. Unless there had been a very marked masking of UV absorption in the range 260 to 300 mμ, as a result of a complex formation, the extracts were RNA-phosphorus free.

Humic associated phosphorus was shown to occur in Egmont soil (41). Phosphorus recovered in this fraction accounted for 30 percent of the total extracted organic phosphorus. There is little knowledge of the type of compounds likely to occur in that fraction. Anderson (4) showed the occurrence of DNA components in the acid insoluble fraction, but quantitatively the amount was a very low proportion of the total fraction. Dormaar (26) has studied the humic associated phosphorus in several Canadian soils by electrophoresis and has shown that several distinct patterns occur, associated with the different soil types. No suggestion has been made as to the type of compounds involved, except that the patterns did not correspond to DNA, RNA or phytic phosphorus.

The complex nature of soil organic phosphorus has been shown very clearly. The nature of mobile organic phosphorus, the problem of concern, could be equally complex.

MATERIALS AND METHODS

Collection and Preparation of Soil Material

Soil material was collected from the B_{Ca} horizon of a Mohave sandy loam which is located northwest of Tucson. The soil material from the B_{Ca} horizon is gravelly and calcareous. The soil was air dried, rolled, sieved through a 2 mm. sieve and thoroughly mixed to give as homogeneous a sample as possible.

The soil was found to contain 1.5 percent equivalent of calcium carbonate, 0.11 percent organic matter, and to possess a cation exchange capacity of 12 Meq/100 gm. The exchange complex was mainly saturated with calcium and the pH value of the soil paste was 7.6.

Preparation of Soil Columns

Columns were constructed by cementing a plastic screen on the base of a plastic tube having a 4-inch inside diameter and a 12-inch length. A filter paper was placed on top of the plastic screen to retain the soil particles. The column was filled to a depth of 8 inches with the air dry Mohave sandy loam which represented about 2400 grams of soil material per column. The moisture equivalent of a packed column was 610 ml./column and the percolation rate was 354 ml./hr./column

Materials Added to Soil Columns

Treatment of a soil column with an energy source such as sucrose was previously found to increase the amount of phosphorus moving with the soil-column-solution (34). In a preliminary study one soil column was treated with sucrose and ammonium nitrate in order to develop appropriate methods for physical and chemical fractionation of mobile phosphorus compounds.

After developing a suitable method, a set of six soil columns was prepared as previously described. Duplicate columns were treated with sucrose, plant residue and no material, (Table 1). The materials were mixed with the top 2 inches of soil, which was then saturated with water. The soil columns were incubated for a week at room temperature. Twenty ml. of water were added every other day to keep the treated area of the soil wet. After incubation, the soil solutions were then displaced once each 4 days, for a total of 6 displacements, by pouring 200 ml. of deionized water into the top of each column. The reason for using 200 ml. was to facilitate movement of phosphorus in amounts convenient for analysis. The displaced soil solutions were brought to volumes of 200 ml. each and then stored in the refrigerator prior to analysis to reduce microbial activity to a minimum.

Analysis of Displaced Soil Solution

Determination of Inorganic Phosphorus

The method of Pons and Guthrie (49) was employed for the determination of inorganic phosphate in the presence of organic phosphate. The transmission of the blue isobutanol layer was measured at 630 mμ using a Beckman spectronic "20" colorimeter. The pH values of the samples were adjusted, before adding the molybdate reagent, to a pH value around 4 with Dinitrophenol as an indicator. Dinitrophenol being colorless at pH values below 4 did not interfere with the method.

Since the formation of the phosphomolybdate complex required the presence of excess sulfuric acid (49) hydrolysis of organic phosphorus if present might take place. It seemed important to test some organic phosphates for possible interference.

The percentage of hydrolysis of some organic phosphates was determined as shown in Table 2. Pons and Guthrie (49) reported that hydrolysis values for glucose-1-phosphate ranged from 0.13 to 2.87 percent depending on the period from the time the molybdate was added to the time at which the shaking with stannous chloride was completed. Their reported value of 0.97 percent hydrolysis for a period of 28 minutes is comparable to the value of 1.21 percent, Table 2, for G-6-P. For a set of 12 samples it took an average of 30 minutes from the time the molybdate reagent was added to the

Table 1. Treatments of Soil Columns

Material Added	Amount of Material	
	Tons/acre	g./column
None	--	--
Bean residue	10	27.40
Sucrose and NH_4NO_3	9.5*	26.03
	0.5**	1.38

* carbon is equivalent to that of 10 tons bean residue.
** nitrogen is equivalent to that of 10 tons bean residue.

Table 2. Hydrolysis Products of Some Organic Phosphates

Organic phosphate	Total P in sample	Inorganic P detected	Hydrolysis
	ug		%
ATP	40	0.95	2.37
PEP	38	1.75	4.60
G-6-P	70	0.85	1.21

time at which the blue isobutanol layer was separated.

The use of Millipore filtrates rather than direct use of soil solution, in determining inorganic phosphorus, was preferred in order to avoid the interference of organic phosphates.

Determination of Total and Organic Phosphorus

Total phosphorus was determined in the same way as inorganic phosphorus after digesting the samples with a mixture of nitric and perchloric acids. Dry ashing was used instead of wet digestion for determining total phosphorus in KOH extracts where precipitation of $KClO_4$ would otherwise take place. When wet digestion was compared with dry ashing, comparable recovery of total phosphorus was obtained. Values of total phosphorus for two equal amounts of lecithin were 4.50 and 4.48 µg for wet digestion and dry ashing respectively.

Organic phosphorus was calculated by subtracting values of inorganic phosphorus from those of total phosphorus.

Physical Fractionation of Mobile Soil Phosphorus

Centrifugation

The use of centrifugation was compared with the use of Millipore filtration for the purpose of fractionating phosphorus carrying particles according to size. Ultracentrifugation proved to be unsatisfactory due to reaction of centrifugation-tube-caps with some

basic samples. The Millipore filters, on the other hand, had the advantage of separating fractions of definite particle sizes. For these reasons the use of Millipore filters was preferred.

Millipore Filtration

Inorganic particles, microbial cells and cellular debris were separated into three fractions according to their sizes. Particles larger in diameter than 0.45 μ were separated by Millipore filters of 0.45 μ in pore diameter (type HA). The fraction separated in this way was designated as "R_1". Particles that ranged in diameter from 0.45 μ to 10 mμ were then separated by Millipore filters of 10 mμ in pore diameter (type VF). Such fraction was designated as "R_2". The fraction that passed both filters, or the filtrate was designated as "F".

Inorganic phosphorus, as mentioned before, was determined in this filtrate rather than in the original soil solution. Total phosphorus was determined in all fractions ("R_1", "R_2" and "F").

Microscopic Examinations

Slides, one representing each treatment, were prepared by spreading and drying drops of the soil solutions. They were then examined under a light microscope in order to identify the colloidal particles which may be clay particles, whole microbial cells and cellular debris.

Chemical Fractionation

The method developed by Martin (39) for extracting and fractionating soil phosphorus was employed. Briefly, the method was based on extraction by a 0.3M KOH solution followed by a partial fraction of the KOH-extract through anion exchange resin column. Detailed discussion of the method follows and Figure 1 presents a schematic view of the fractionation sequence.

Solvent Extraction

In the main experiment, the original soil solution was made 0.3M in KOH by adding 6M KOH. The extraction was repeated two more times, each time with 25 ml. of 0.3M KOH solution. Filtration of the extracts were made through base-resistant Millipore filters of 1.5 μ in pore size (type OH). The reason for using the original soil solutions and not the fraction R_1 was to include the other physical fractions R_2 and F. The 3 KOH extracts were pooled and brought to 200 ml. volumes by adding 0.3M KOH solution.

Extraction with lipid solvents, a mixture of ether, chloroform, ethyl alcohol and acetone (33), was not successful following base extraction due to the fact that phospholipids such as lecithin dissolve in base.

Total base insoluble phosphorus, remaining on the filters after the series of extractions, was determined. Both inorganic and

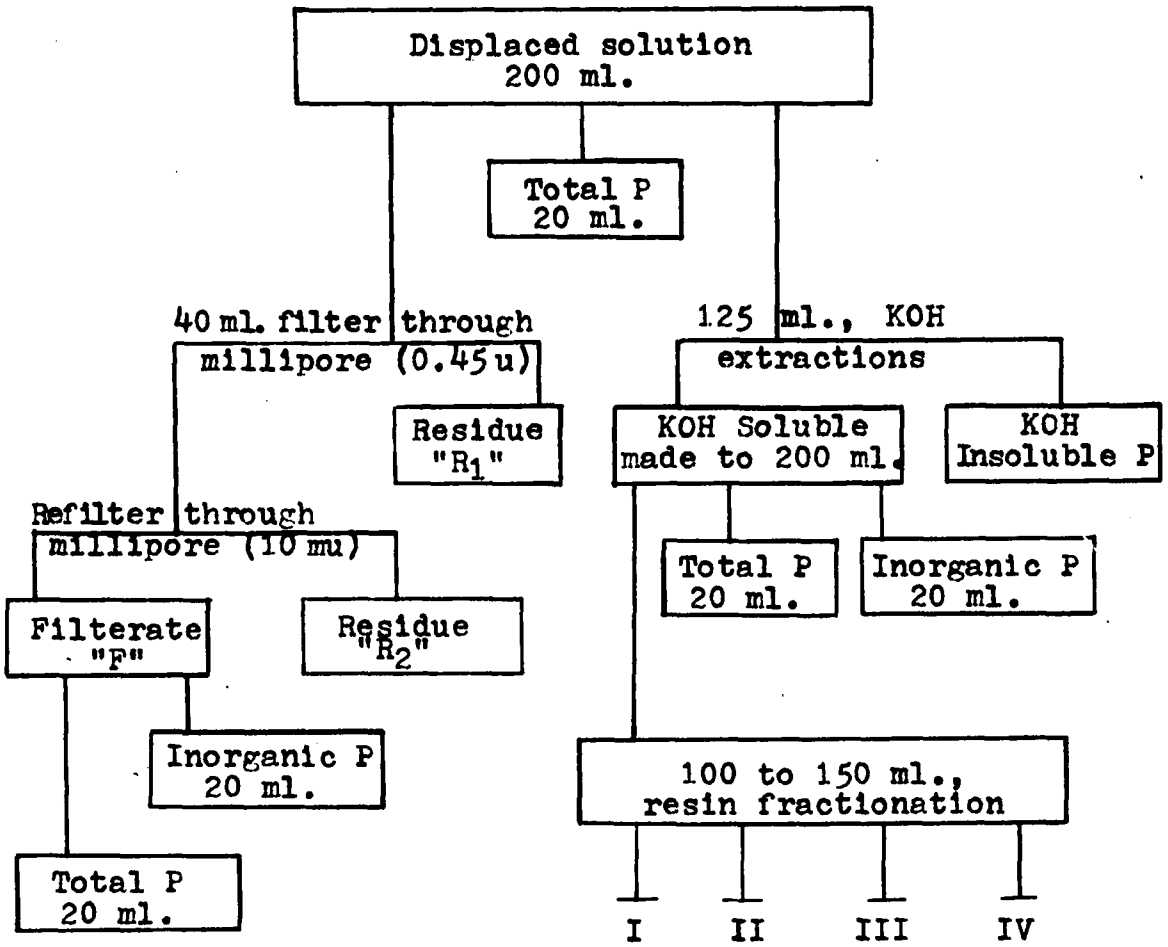

Fig. 1: Phosphorus fractionation procedure in solutions displaced from soil columns

total phosphorus were determined in the 0.3M KOH extract. Total phosphorus was determined using the dry ashing procedure as mentioned before.

Anion Exchange Chromatography

Materials: 1. Chloride saturated AG 1-X4 resin,[1] 50-100 mesh was used. This resin is similar to Dowex 1 but more refined.

2. Calcium phytate (California Corporation for Biochemical Research) was put into solution as follows. One-tenth gram Ca-phytate was converted to phytic acid by suspending it in H_2O and adding Dowex 50 W X-8 resin, H form. After removing the resin the phytic acid was converted to K-phytate and then brought to 500 ml. volume by adding 0.3M KOH solution.

3. Ribose nucleic acid and lecithin (Nutritional Biochemical Company) were readily dissolved in 0.3M KOH solution.

4. Other organic phosphates, like ATP, AMP, G-6-P and PEP also were dissolved in 0.3M KOH solution.

Preparation of the Resin Column and Fractionation Procedure: A column, 12.5 x 1 cm. after settling, was prepared from

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[1]Also known as De Acidite FF, and it is a Bio-Rad product.

a slurry of AG 1-X4 (50-100 mesh) Cl-form resin. A light plug of glass wool was placed on top of the resin. Fifty ml. of H_2O were passed through the column followed by 80 ml. of 0.3M KOH solution. The elutes were discarded. The 0.3M KOH extract followed immediately at a flow rate of 1 ml./min. The sample was washed through with 20 ml. of 0.3M KOH and combined with the first elute to form fraction I. Subsequent elution with 50 ml. of 0.85M KOH yielded fraction II, and then fraction III was eluted with 25 ml. of 2M KOH solution. Finally 25 ml. of 10 percent HCl was passed through the column to yield fraction IV. A fresh resin sample was used each sequential elution.

The fractions were analyzed for total and inorganic phosphorus within one day and were stored in a refrigerator prior to analysis.

Fractionation of known phosphate compounds: The behavior of inorganic phosphorus (KH_2PO_4), ribose nucleic acid (RNA), adenosine tri-phosphate (ATP), adenosine mono-phosphate (AMP), phytin, glucose-6-phosphate (G-6-P), phospho enol pyruvic acid (PEP) and lecithin on AG-1-X4 (50-100 mesh) resin column was tested. The recoveries of these compounds in the four different fractions are reported in Table 3 as amounts of total phosphorus. Lecithin, G-6-P, PEP and

Table 3. Fractionation of Known Phosphorus Compounds on AG 1-X4 Resin

Phosphorus compound added to the column	Amount of phosphorus in the added compound	Recovery of phosphorus in the four fractions					% of underlined figures*
		I	II		III	IV	
		0.3M KOH	0.85M KOH		2.0M KOH	10% HCl	
			Total	Inorganic			
	μg						%
Blank (1)	--	1.2	2.0	--	4.5	1.2	--
(2)	--	1.5	2.5	--	4	1.5	--
KH_2PO_4	50	3.5	51	51	6	2	97
G-6-P	117	2	92	--	25	6	77
PEP	50	3	46	--	7.5	3	87
Lecithin	450	28	350	--	54	27	77
	90	2.5	85	--	6.5	4	92
Phytin	80	6	15	9	66	3.0	77
RNA	170	5	30	15	14	136	79
AMP	192	3	35	22	15	150	78
ATP	76	2	16	12	6	62	80

* Underlined figures represent the major fractions of phosphorus.

inorganic phosphate were recovered in good yield in Fraction II. Phytin was recovered in good yield in Fraction III. RNA, AMP and a large part of ATP phosphorus were recovered in Fraction IV. Martin (39) recommended a load of 100 to 200 µg phosphorus on each column. The yield of lecithin in Fraction II was better when an amount of 90 µg lecithin-phosphorus was used instead of 450 µg, Table 3.

UV Spectrum

Nucleic acids and nucleotides are known to absorb light in the ultraviolet region especially in the range 300-260 mu. Absorption spectra for all resin fractions of certain soil solutions were made and compared with the spectra of RNA, ATP and certain other organic phosphates.

RESULTS AND DISCUSSION

Movement of Total, Organic and Inorganic Phosphorus

Results of the preliminary experiment, in Table 4, showed the effect of adding sucrose + NH_4NO_3 in accelerating the downward movement of phosphorus in a soil column. The maximum rate of movement took place in the third displacement which contained the highest concentration of phosphorus. The recovery of phosphorus in the displaced soil solution increased three times from the first displacement to the third and started to decline on the fourth, reaching a minimum on the sixth displacement.

It is of interest to notice that there was relatively little or no change in the movement of inorganic phosphorus from one displacement to the other. The amount of organic phosphorus, on the other hand, followed very closely the changes in total phosphorus movement. The percentage of organic phosphorus recovered in the third displacement constituted 96 percent of the total phosphorus in that displacement while the percentage in the sixth displacement was only 80 percent of the total.

Similar results were obtained from the sucrose treated soil columns in the main experiment except that the maximum rate of movement occurred in the second displacement and not in the third. The

Table 4. Phosphorus Distribution in Some Fractions of the Displaced Soil Solutions of a Sucrose-treated Soil Column (Preliminary Experiment)

Fraction	Phosphorus in Displacement					
	1	2	3	4	5	6
	ug					
Total P	224	350	640	434	300	180
Inorganic P	20	24	26	24	26	20
Organic P	204	326	614	410	274	160
Centrifugation* pellet	132	226	520	302	156	88
Ultra centrifugation+ pellet - P	38	--	50	72	96	47
KOH soluble#: Total P	120	180	445	260	130	65
Inorganic P	32	45	80	52	35	25
Lipid solvents#: Soluble P	7	20	12	16	8	6
Residual P#	10	25	40	20	20	15
Resin fractions: I	--	--	40	20	--	--
II	--	--	122	75	--	--
III	--	--	95	57	--	--
IV	--	--	202	113	--	--

* Centrifugation speed was 25, 000 g.
+ Centrifugation speed was 100, 000 g.
# Centrifugation pellet was the fraction used for KOH extraction and phospho-lipid extraction.

movement of total, organic and inorganic phosphorus in sucrose treated columns as compared with that in bean residue treated columns and that in the untreated ones are shown in Tables 7 through 12. Phosphorus recovered in a total of six displacements is shown in Table 13. Sucrose + NH_4NO_3 treatment resulted in the highest movement of total and organic phosphorus followed by the bean-residue treatment and the untreated. Total movement of phosphorus was increased by the sucrose treatment 8 times and by the bean-residue treatment 5 times over that of the untreated soil columns.

Inorganic phosphorus movement, due to the added carbon sources, was increased only about 30 percent whereas organic phosphorus increased 11 and 8 folds for sucrose and bean residue applications, respectively. It can be seen in Figure 2 that movement of organic phosphorus, from sucrose-treated soil columns, follows very closely that of the total phosphorus; curves for both total and organic phosphorus show exactly the same trend. Similar observation can be seen in Figure 3 for the bean-residue treatment. Curves for movement of inorganic phosphorus occupy low positions in both Figures 2 and 3, and show small change if any. In the untreated columns, from which the movement of phosphorus was generally low, organic phosphorus represented only 43 to 68 percent of total phosphorus recovered in the displaced solutions with an overall average of 55 percent for six displacements. Even though the percentage recovery of organic

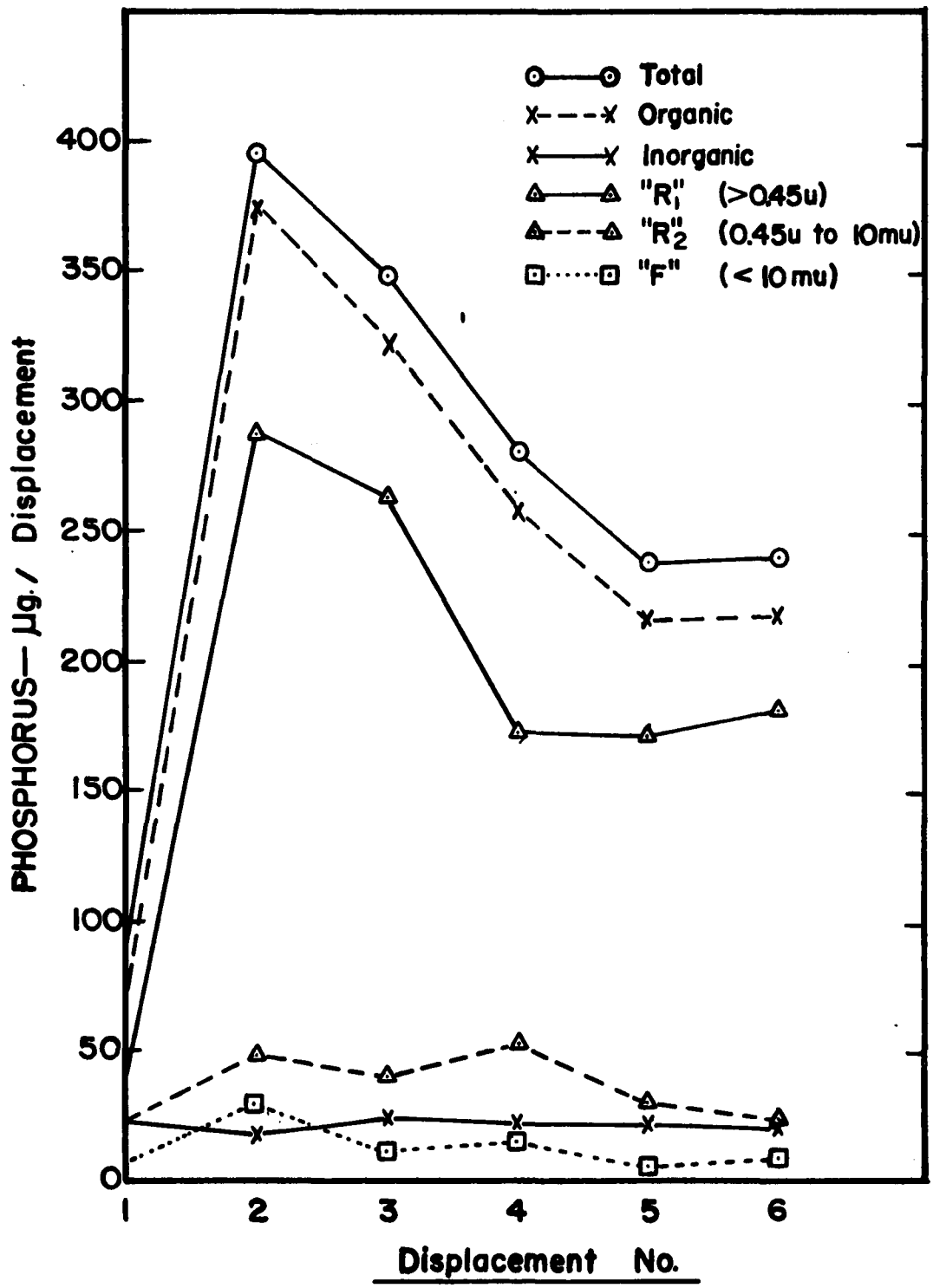

Fig. 2 Size fractions of phosphorus in the displacements of sucrose treated soil columns, (duplicates mean).

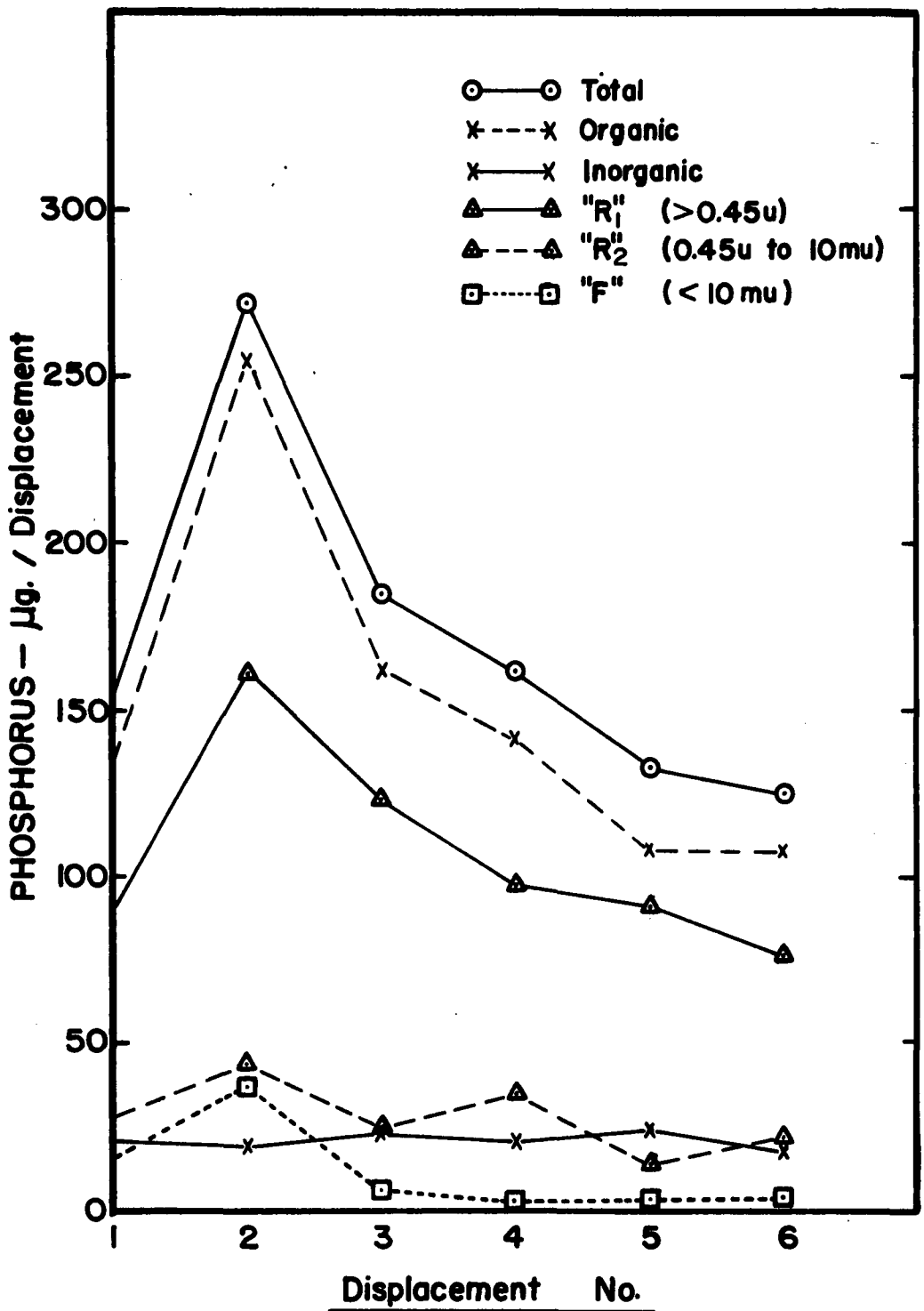

Fig. 3 Size fractions of phosphorus in the displacements of bean residue treated soil columns, (duplicates mean).

phosphorus from the untreated soil columns was quite low, when compared with the treated columns, almost identical curves for the movement of total and organic phosphorus are observed in Figure 4. The curve for organic phosphorus follows the same trend of that of the total phosphorus but at a relatively lower position. Inorganic phosphorus did not account for much of the change in the phosphorus movement. Amounts and forms of mobile phosphorus, for a total of six displacements, are shown in Figure 5.

The presented data demonstrated the predominance of organic phosphorus in the displaced soil solutions when organic matter is available in the calcareous Mohave soil. The extensive increase in phosphorus movement down the soil column, due to treatment with an energy source such as sucrose or plant residue, agrees with the finding by Hannapel et al. (34, 35) who related the increase in recovery of organic phosphorus, in the soil solutions of a Tucson sandy loam, to the increase in microbial activity upon such treatments. It should be mentioned that the differentiation between organic and inorganic phosphorus was based on the method of Pons and Guthrie (49) which detects only the orthophosphate ion in solution. Any inorganic phosphorus other than that in true solution cannot be included. For this reason the term water-soluble was given to inorganic phosphorus determined directly in the displaced soil solution so as to differentiate between that fraction and the KOH-soluble inorganic phosphorus which will be discussed later.

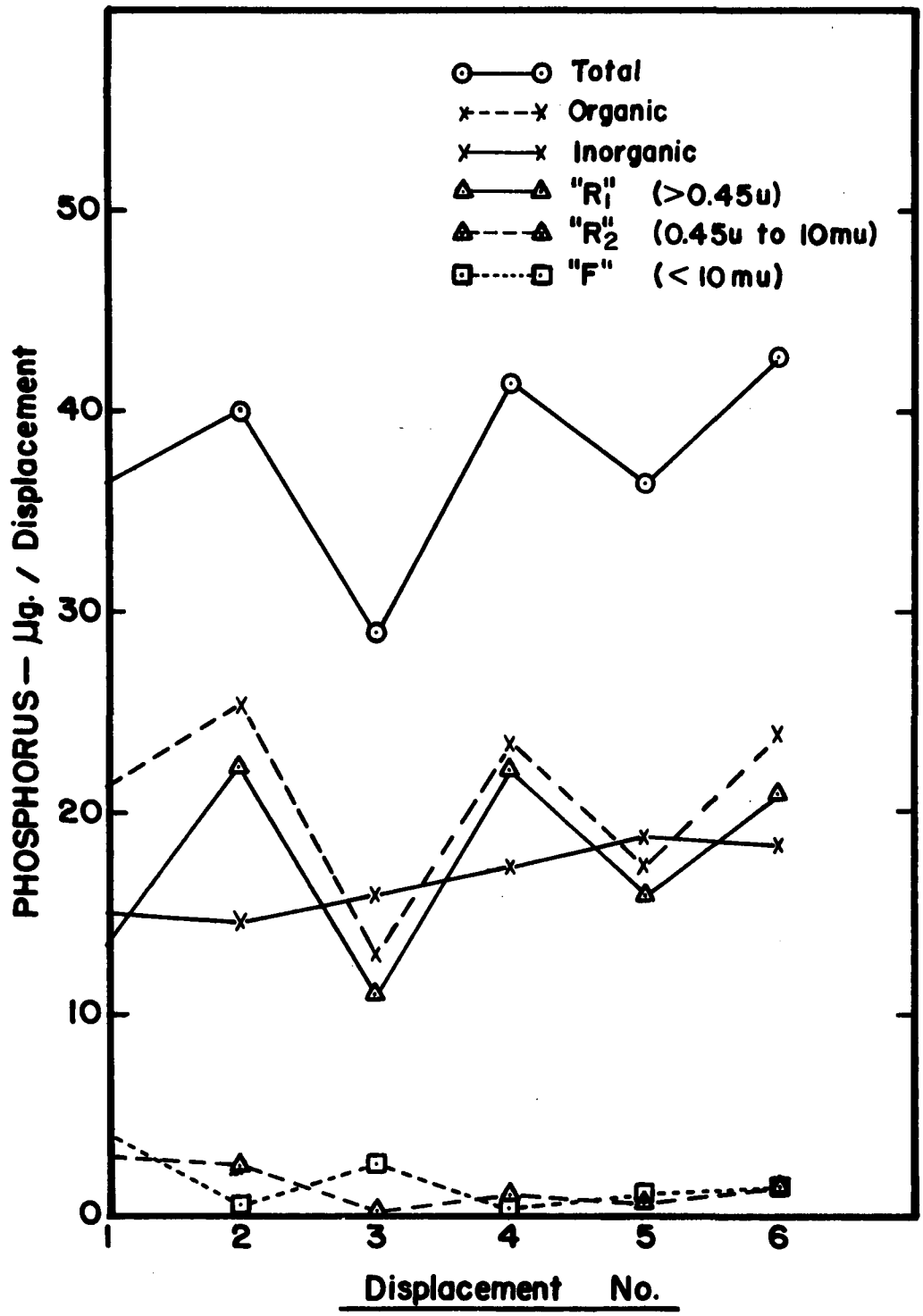

Fig. 4 Size fractions of phosphorus in the displacements of non-treated soil columns, (duplicates mean).

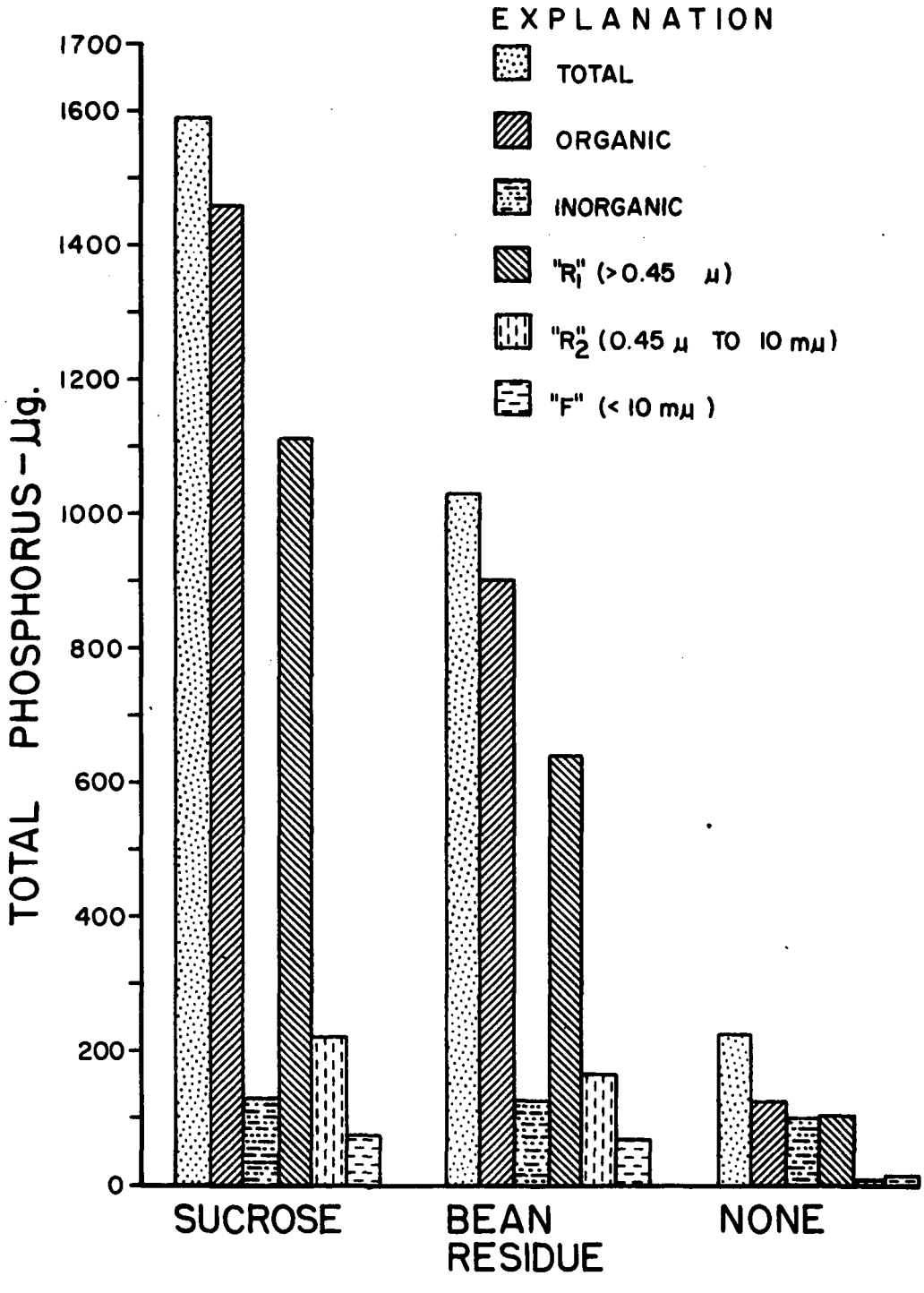

Figure 5.--Size fractions of phosphorus in a total of six displacements.

The Physical Nature of Mobile Phosphorus Forms

The principle means for physical fractionation of the phosphorus in the displaced soil solutions was by Millipore filtration. However, the results of the preliminary experiment in Table 4 were obtained through centrifugation and ultra centrifugation. The size fractions of mobile phosphorus compounds are shown in Tables 7 through 13. The main part of mobile phosphorus was associated with the "R_1" fraction which was separated by 0.45μ Millipore filters. Seventy percent of the phosphorus recovered from the sucrose-treated columns was associated with the "R_1" fraction. Phosphorus in this fraction accounted for 62 percent of the total phosphorus movement from the bean residue-treated columns, Table 5. In the untreated, "R_1"-phosphorus represented 50 percent of the total. However, this same amount represented 90 percent of organic phosphorus recovered from the untreated columns.

A large portion of phosphorus that passed through the 0.45μ Millipore filters was retained by a 10 mμ filter. The resulting fraction which ranged in particle size from 0.45μ to 10 mμ was given the symbol "R_2". Phosphorus in the "R_2" fraction was as high as 15 percent of the organic phosphorus for the sucrose treatment and 18 percent for the bean residue treatment. The phosphorus content of this fraction was not detected in most of the displacements from the untreated soil. It was calculated therefore by difference.

Table 5.* Percentage Distribution of Phosphorus in the Size Fractions of a Total of Six Displacements

Treatment	Phosphorus--percent of the total					
	Water soluble inorganic	Organic	R_1 (>0.45μ)	R_2 (0.45μ to 10 mμ)	F (<10 mμ) Total	F (<10 mμ) Organic
	%					
None	44.5	54.5	47.2	3.5	49.0	4.9
Sucrose + NH_4NO_3	8.2	91.8	70.2	13.6	13.0	4.8
Bean Residue	12.2	87.8	62.4	16.0	18.8	6.6

*Calculated from Table 13 in the Appendix.

All the water soluble inorganic phosphorus passed both filters and appeared in the filtrate which was given the symbol "F". Particles in this fraction did not exceed 10 mu in size. Such filtrates probably were close to being a true solution. The amount of organic phosphorus falling in this fraction was quite low, representing values of only 9, 8 and 5 percent of organic phosphorus for untreated, bean residue and sucrose treated soil columns, respectively. The distribution of organic phosphorus between the three fractions is shown in Figures 2, 3 and 4. Curves for R_1 fraction follow the pattern of those of total and organic phosphorus.

It can be noticed in Tables 7 through 13 that the sum of phosphorus determined in the three size fractions, "R_1", "R_2" and "F", was not exactly equal to total phosphorus in most of the cases. The average recovery in the size fractions of the treated columns was about 97 percent. Hannapel et al. (34) reported an average recovery for a similar study of 70 percent and they explained the discrepancy on the basis of incomplete oxidation of some fractions. The reason for the higher recovery in the present study was probably due to the use of smaller samples for oxidation, Figure 1. Martin (39) reported a considerable loss on oxidation with $HClO_4$ when the samples used contained more than 6 µg of organic phosphorus.

In the light of these results it appeared that more than 90 percent of organic phosphorus, moving with the soil solution, was particulate

in nature and only 10 percent or less of it was in true solution.

The main portion of the organic phosphorus was associated with colloidal particles larger in size than 0.45μ and a considerable portion of it was associated with particles ranging in size from 0.45μ to 10 mμ. It was thought that these colloidal particles were mostly microbial in nature, consisting of cells and cellular debris. Microscopic examination of some displacements proved the existence of microbial cells in the soil solution especially in those recovered from treated soil columns. Some clay particles and other debris appeared in the solution but microbial cells, especially those of bacteria, were predominant in the slides examined. The association of organic phosphorus, moving with the soil solution, with the microbial fraction was also discussed by Hannapel et al. (34, 35).

Organic phosphates associated with microbial cells and debris would not be expected to behave in a manner similar to that of free molecular organic phosphates. Being physically different, the mobile soil-organic-phosphates would not be as available to plants as the molecular organic phosphates. As previously discussed (48, 50), free organic phosphates such as phytin, lecithin, nucleic acids, nucleotides and glycerophosphates were available to plants while organic phosphates present in soil solutions were not. It is thought that such difference in availability to plants was primary, due to the difference in the physical disposition; mobile organic phosphorus being particulate while the other

tested phosphates were molecular and water soluble.

The chemical fractionation in the following section adds further proof to the connection between the mobile organic phosphorus and the microbial cells.

Partial Chemical Fractionation

A large portion of phosphorus moving with the soil solution could be extracted with 0.3M KOH solution. The amounts of KOH-extractable phosphorus, reported in Tables 14 through 19, represented 80 to 87 percent of total phosphorus in solutions. Average percentage of KOH-extractable phosphorus for the untreated, bean residue-treated and sucrose-treated columns was 85, 82 and 81 percent respectively. The residual or KOH-non-extractable phosphorus accounted for the remaining 15 to 19 percent of total phosphorus, Table 6.

The chemical nature of the residual phosphorus was not thoroughly studied. However, an appreciable amount of this phorphorus could be extracted with lipid solvents, such as a mixture of ethanol, ethyl ether, acetone and chloroform (33). The amounts of phosphorus extracted by these solvents ranged from 6 to 20 µg per displacement for a sucrose treated column, Table 4. These values accounted for 2 to 8 percent of total phosphorus present in the centrifugation pellet, the fraction used for the extraction. Phosphorus compounds extracted this way were assumed to be phospholipids but no further work was

Table 6.* Percentage Distribution of Phosphorus in the Chemical Fractions of a Total of Six Displacements

Treatment	Phosphorus--percent of the total						
			In the resin fraction				
	KOH insoluble	KOH soluble	I	II		III	IV
				Total	Inorganic		
	%						
None	14.2	84.9	2.7	55	51.9	10.0	13.3
Sucrose + NH_4NO_3	17.4	80.8	6.2	20.7	14.9	23.1	27.7
Bean Residue	16.2	81.7	7.2	29.5	20.6	17.7	23.4

*Calculated from Table 20 in the Appendix.

directed to that portion of phosphorus. It should be mentioned that phospholipids such as lecithin and glycerophosphates were dissolved in base. Thus the amounts of phosphorus extracted with lipid solvents, following the KOH extraction, probably represented only a portion of phospholipid. Bartholomew and Goring (8) were able to extract phospholipid fraction from microbial cells with a similar solvent mixture but following acid extraction rather than the base extraction used in the present study. They estimated the phospholipid phosphorus to be 2.8 to 10.1 percent of total phosphorus present in cells; the percentage decreasing by increasing the incubation period.

Bartholomew and Goring (8) also pointed to the possibility of removing from solution of some phosphorus bearing complexes of high molecular weight upon addition of 1 percent bentonite clay; complexes that would be extracted otherwise by water or trichloracetic acid. Such removal might have occurred in the KOH extracts of the displaced soil solutions and accounted for some of the KOH-insoluble phosphorus since the presence of some colloidal clay particles was observed during the microscopic examination.

The KOH-extractable phosphorus was studied further by column chromatography. The KOH extracts were separated into 4 fractions through resin columns of the type AG-1, X4. Fraction I was that fraction which was not retained by the resin column and was eluted through with 0.3M KOH solution. Fraction II, fraction III and then fraction IV

were eluted with 0.85 M KOH, 2 M KOH and 10 percent HCl solutions, respectively. The distribution of phosphorus between these fractions is shown in Tables 14 through 19 and the behavior of some known organic phosphates, when added to resin columns, is presented in Table 3.

Phosphorus falling in fraction I of the displaced soil solutions did not account for more than 9 percent of the total KOH-extractable phosphorus in any treatment, Table 20. None of the tested organic phosphates yielded any appreciable amount of phosphorus in this fraction. Only few ug. of phosphorus were detected in fraction I except when the load of organic phosphate on the resin column was relatively high, Table 3. Martin (40) identified phosphorus of this fraction as humic acid-associated phosphorus. However, any phosphate complex that could not be retained by the resin would likely fall in this fraction. Martin (39) observed a considerable decrease in the amount of phytin retained by resins of high cross linking (AG-1, X8) when it was added to KOH extracts of soils. He related that to a complex formation upon the addition of phytin to KOH extracts. The degree of retention of phytin within resins of low cross linkage (AG-1, X3-5) was not affected much by mixing phytin with KOH extracts.

If the retention of phytate phosphorus present in KOH extracts was complete within such low cross linked resins as the one used for this study (AG-1, X4), the retention of a more complex organic phosphate such as DNA might not necessarily be as complete. Indeed,

Anderson (4) showed the occurrence of DNA in the acid-insoluble fraction which would likely fall in fraction I, but quantitatively the amount was generally a very low portion of the fraction. Anderson and Hance (6) reported the presence, in alkaline extracts of soils, of a phytin complex containing carbohydrate and proteins. No more knowledge could be obtained about the identity of organic phosphates likely to fall in this fraction.

Phosphorus appearing in fraction II accounted for 25, 36 and 65 percent of the KOH extractable phosphorus for sucrose, bean residues and untreated columns, respectively. The major form of phosphorus appearing in this fraction was inorganic. Inorganic phosphorus accounted for 94, 81 and 72 percent of total phosphorus in fraction II for untreated, bean residue and sucrose treated soil columns, respectively. The percentages of KOH soluble inorganic phosphorus that appeared in fraction II, in Table 6, were almost twice as much as that of water soluble inorganic phosphorus, in Table 5, except for untreated columns where only 10 percent increase in inorganic phosphorus was observed. This increase in inorganic phosphorus upon extraction with alkaline solution is not surprising due to the fact that considerable amount of inorganic phosphorus is retained within the microbial cells and can be released and brought into solution only upon the break-down of the cell membranes. Hydrolysis of some organic phosphates might have occurred but it is thought that the inorganic

phosphorus of microbial cells was the main source for KOH-extractable inorganic phosphorus.

Organic phosphorus of fraction II was not more than 30 percent of the whole fraction for most of the displaced solutions. It also accounted for 3 to 15 percent of total KOH-soluble phosphorus, averaging to 4, 7 and 11 percent for the untreated, sucrose and bean residue treated columns, respectively. The distribution of organic phosphorus in this fraction as compared to the other fractions can be seen in Figures 6, 7 and 8. Totals for six displacements are presented in Figure 9. Glucose phosphates, glycerophosphates and other simple monophosphate esters comprised the main part of organic phosphate of this fraction. As shown in Table 3, inorganic phosphates, G-6-P, PEP and lecithin appeared mainly in this fraction. Traces of nucleotide-phosphorus were also detected in fraction II of the displaced soil solutions. Martin (40) was able to quantitatively recover glycerophosphates in fraction II.

The main part of KOH-soluble organic phosphorus appeared in fractions III and IV, with fraction IV containing more phosphorus for almost all displacements, Figures 6 through 9. Fraction III was identified by Martin (40) to contain the bulk of the acid-soluble phosphorus which would include phosphate esters of low molecular weight likely to be involved in metabolic reactions. Fraction IV could be identified as the RNA nucleotides fraction. Since these two fractions accounted for the major part of organic phosphorus, Tables 14 through

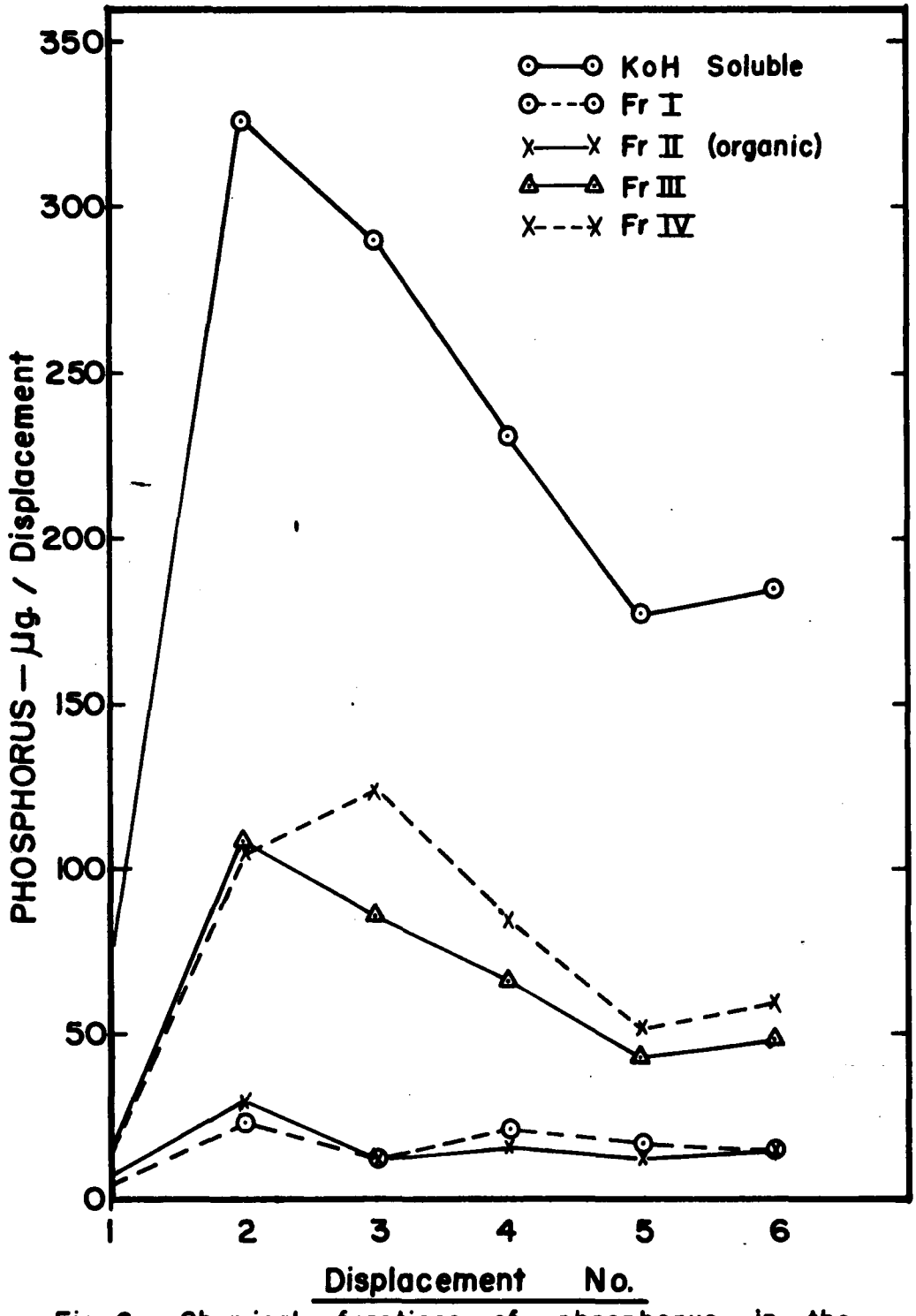

Fig. 6 Chemical fractions of phosphorus in the displacements of sucrose treated soil columns, (duplicates mean).

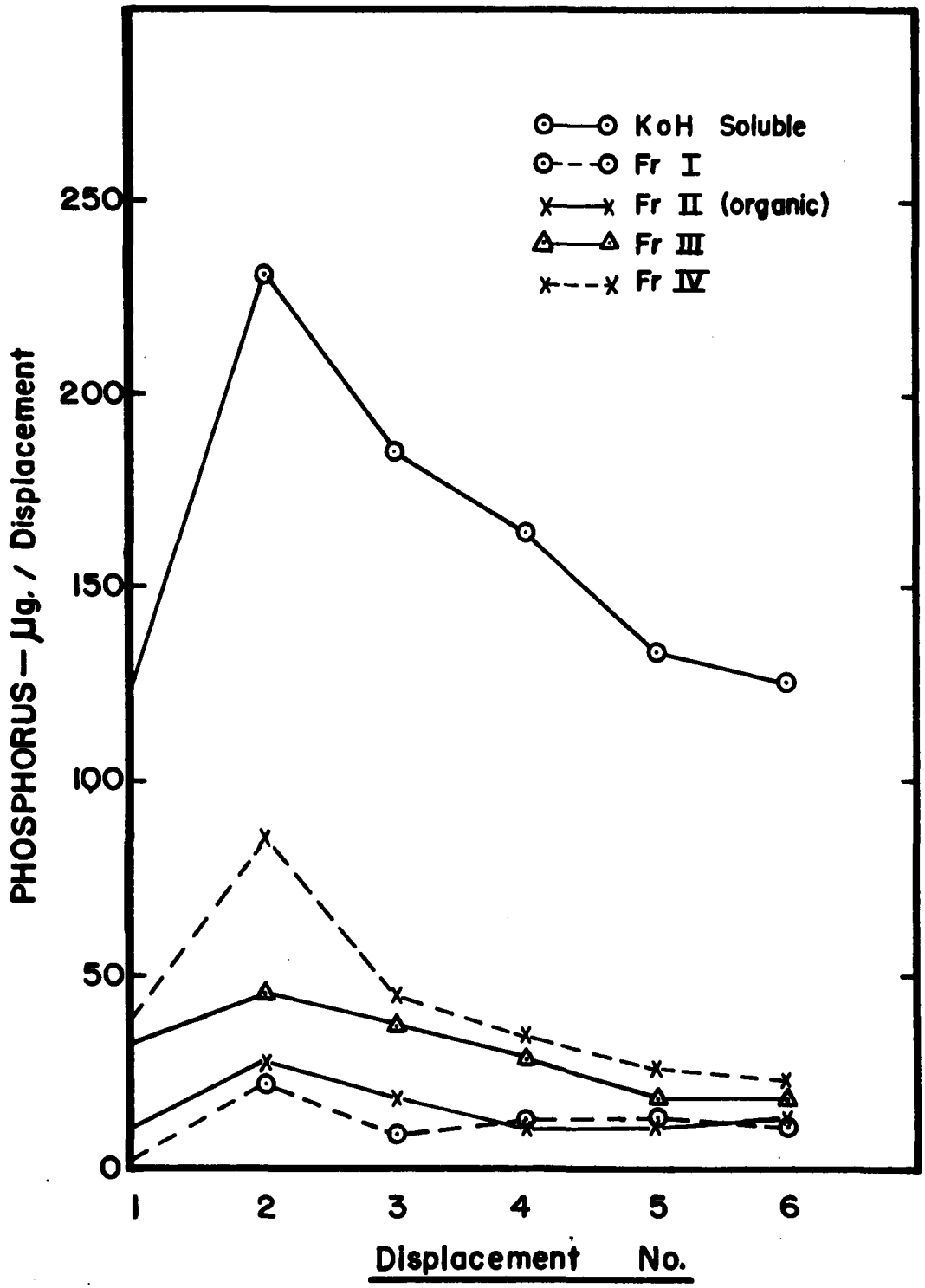

Fig. 7 Chemical fractions of phosphorus in the displacements of bean residue treated soil columns, (duplicates mean).

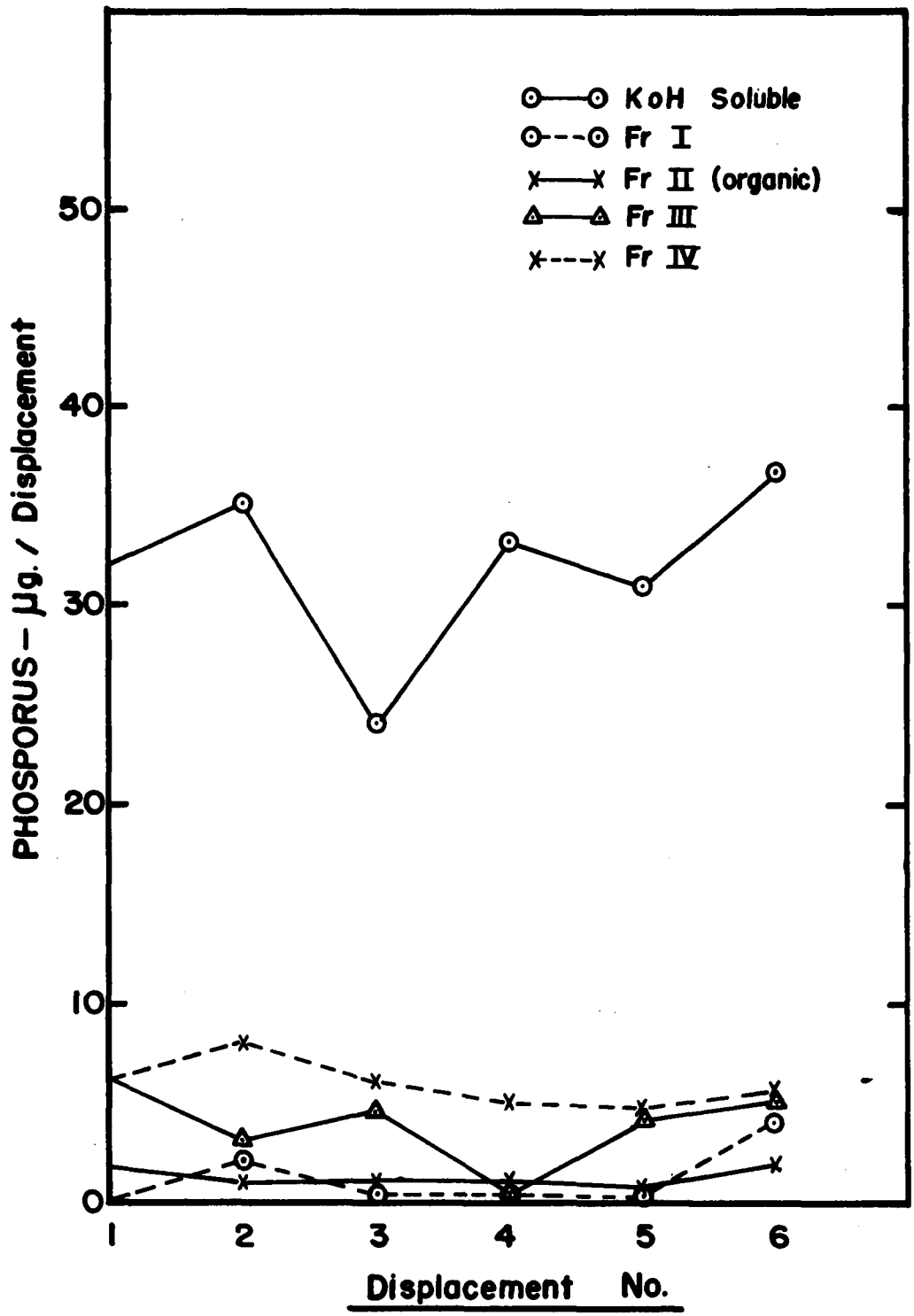

Fig. 8 Chemical fractions of phosphorus in the displacements of the non – treated columns, (duplicates mean).

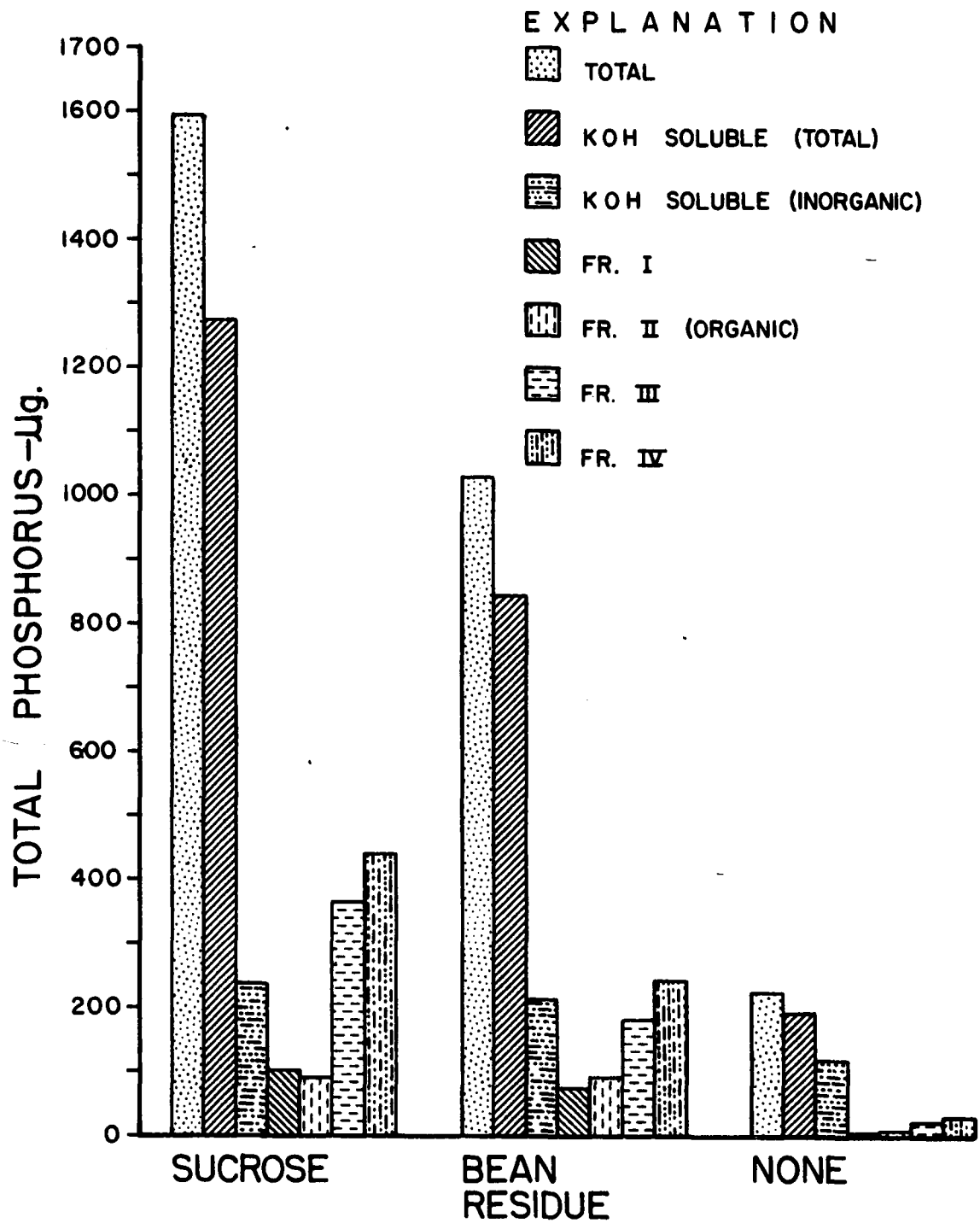

Figure 9.-- Chemical fractions of phosphorus in a total of six displacements.

20, they will be discussed in more detail in the following sections.

The Acid Soluble Phosphorus

From the organic phosphates tested, only phytin appeared mainly in fraction III, Table 3. However, small portions of other organic phosphates especially G-6-P and nucleotide-phosphates appeared also in this fraction.

When the method of Caldwell and Black (14), for precipitating phytin as a ferric salt at pH 1.8, was adopted in an attempt to isolate phytin from fraction III of some displaced solutions, no precipitate could be obtained. It was thought that the failure of precipitate formation was probably due to the low concentrations of phytin in the tested fractions. The highest amount of phosphorus recovered in fraction III was 125 µg per 200 ml displacement, Table 15. Assuming that the major part of this phosphorus was in phytate form, still the concentration of it would not be suitable for successful precipitation. An attempt to precipitate phytate phosphorus from solutions containing 200 µg phytate phosphorus (of commercial source) per 100 ml solution did not succeed either. It can be noticed that such concentration was more than 3 times as much as that in the sample of fraction III that contained the highest amount of phosphorus (125 µg per 200 ml). Martin (40), by adopting the method of Cosgrove (21), which employs HCl gradient elution chromatography, was able to detect considerable

amounts of phytate phosphorus in fraction III of KOH-extracts of some Canadian soils.

Phytate phosphorus and other related compounds probably accounted for a large portion of the phosphorus associated with the acid soluble fraction of the soil solutions.

It is important to note that more than 23 percent of total phosphorus, moving with the soil solution of the sucrose-treated columns, was recovered in the acid soluble fraction which was thought to consist mainly of phytin and phytin-related compounds. The recovery of phytin and other related compounds from sucrose-treated columns, where plant material was either absent or present in insignificant amount, suggests that phytin could be a microbial product.

The RNA Nucleotides Phosphorus and Their Ultra Violet Spectrum

Phosphorus eluted with 10 percent HCl, namely fraction IV, could be attributed to RNA nucleotides. When the behavior of yeast RNA, AMP and ATP was checked with the standard fractionation method, they were recovered in good yields in fraction IV. No other tested compound appeared in this fraction, Table 3. A large part of organic phosphorus present in the KOH extracts of the displaced soil solutions behaved in a way similar to that of RNA nucleotides. The average amounts of phosphorus in this fraction for a total of six displacements were 442, 242 and 30 µg for sucrose, bean residues and

untreated columns, respectively. Such amounts accounted for 42, 39 and 44 percent of the total KOH-extractable organic phosphorus, Figure 9.

When the absorption of some KOH extracts in the ultra violet region was studied, spectra similar to that of yeast RNA were observed. RNA and nucleotide-characteristic absorption appeared to be between 230 and 300 mμ with a maximum absorption at wave length of 260 mμ. Wave lengths of maximum absorption for RNA, DNA and nucleotides have been reported by Walker (59).

As observed in Figures 10, 11 and 12 there were absorption regions around wave length of 240 mμ in the spectrum of KOH extracts. Absorption in that region was very intense to the extent of masking the absorption peak of RNA in some solutions. The source of such absorption is not known, however, RNA and ATP absorbed also in this region but not with the same high intensity observed in soil solution extracts. Whether the compounds absorbing in this region (240 mμ) have any connection with organic phosphates, especially that of nucleotides, is not known.

Another experiment was undertaken in which RNA was added to KOH extracts and then fractionated by the standard method. The ultra violet spectrum was checked for all fractions. Absorption in the characteristic region of RNA took place in almost all fractions except in fraction I, Figure 11, but the intensity of absorption was low in

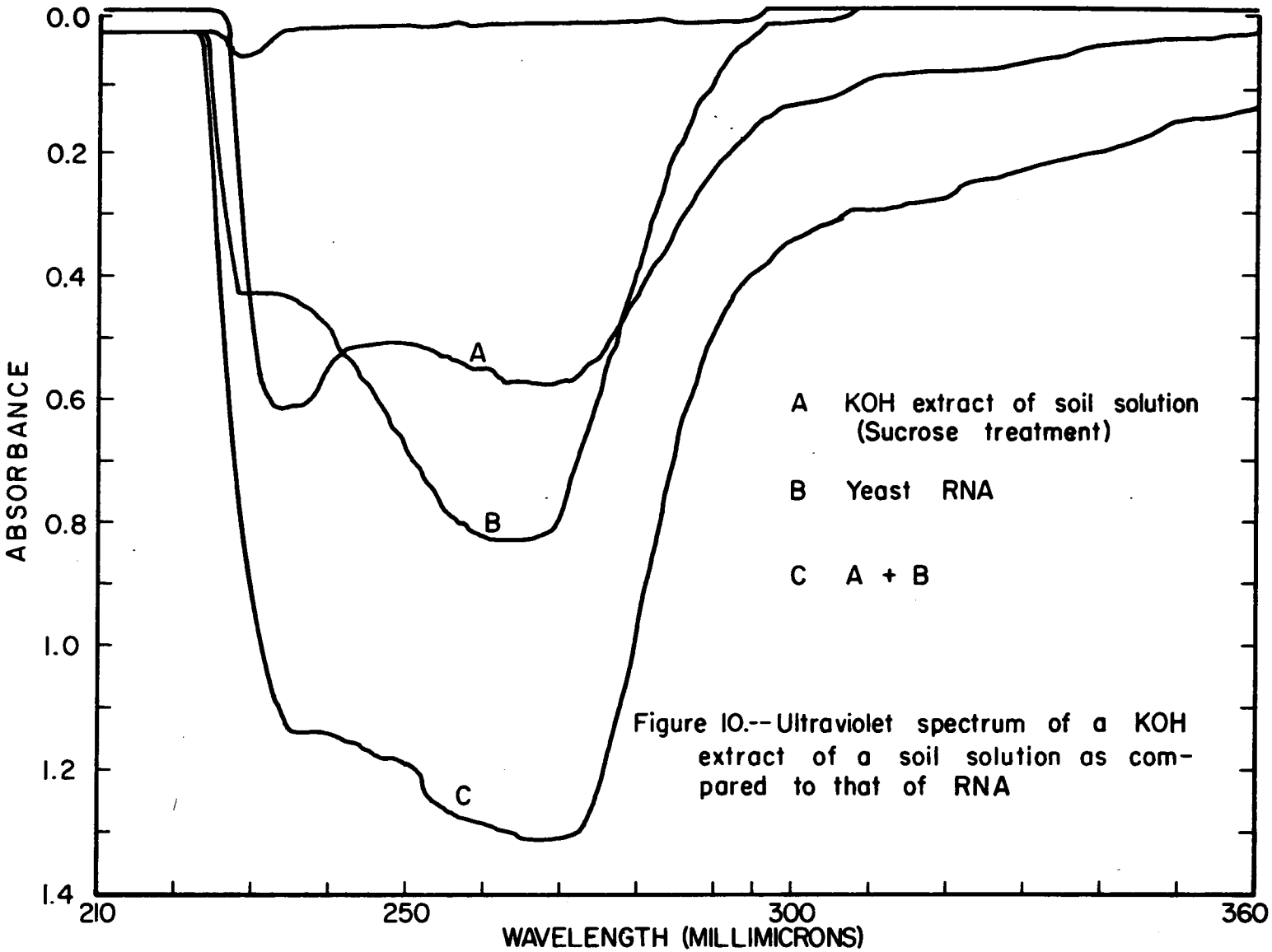

Figure 10.--Ultraviolet spectrum of a KOH extract of a soil solution as compared to that of RNA

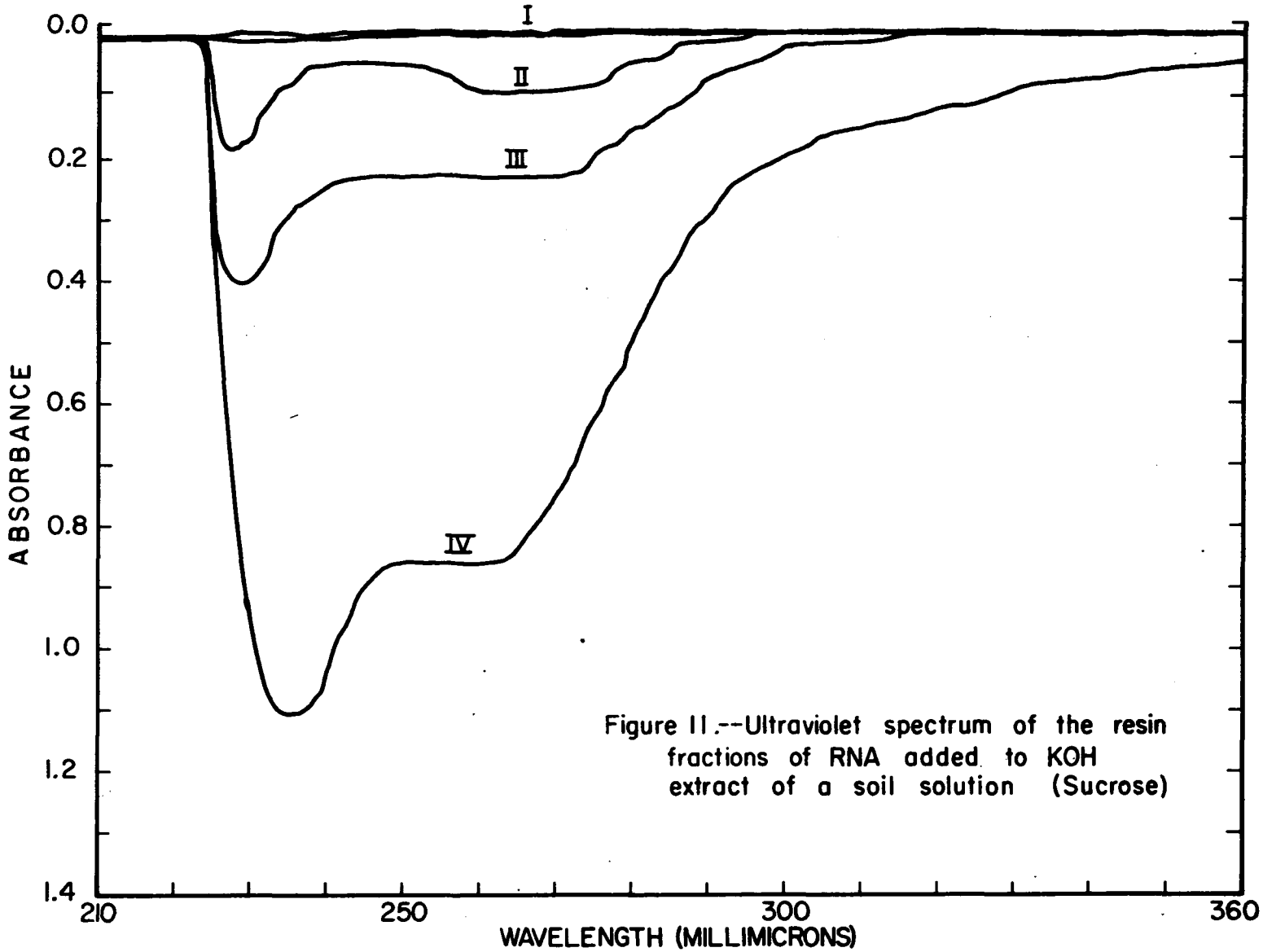

Figure 11.--Ultraviolet spectrum of the resin fractions of RNA added to KOH extract of a soil solution (Sucrose)

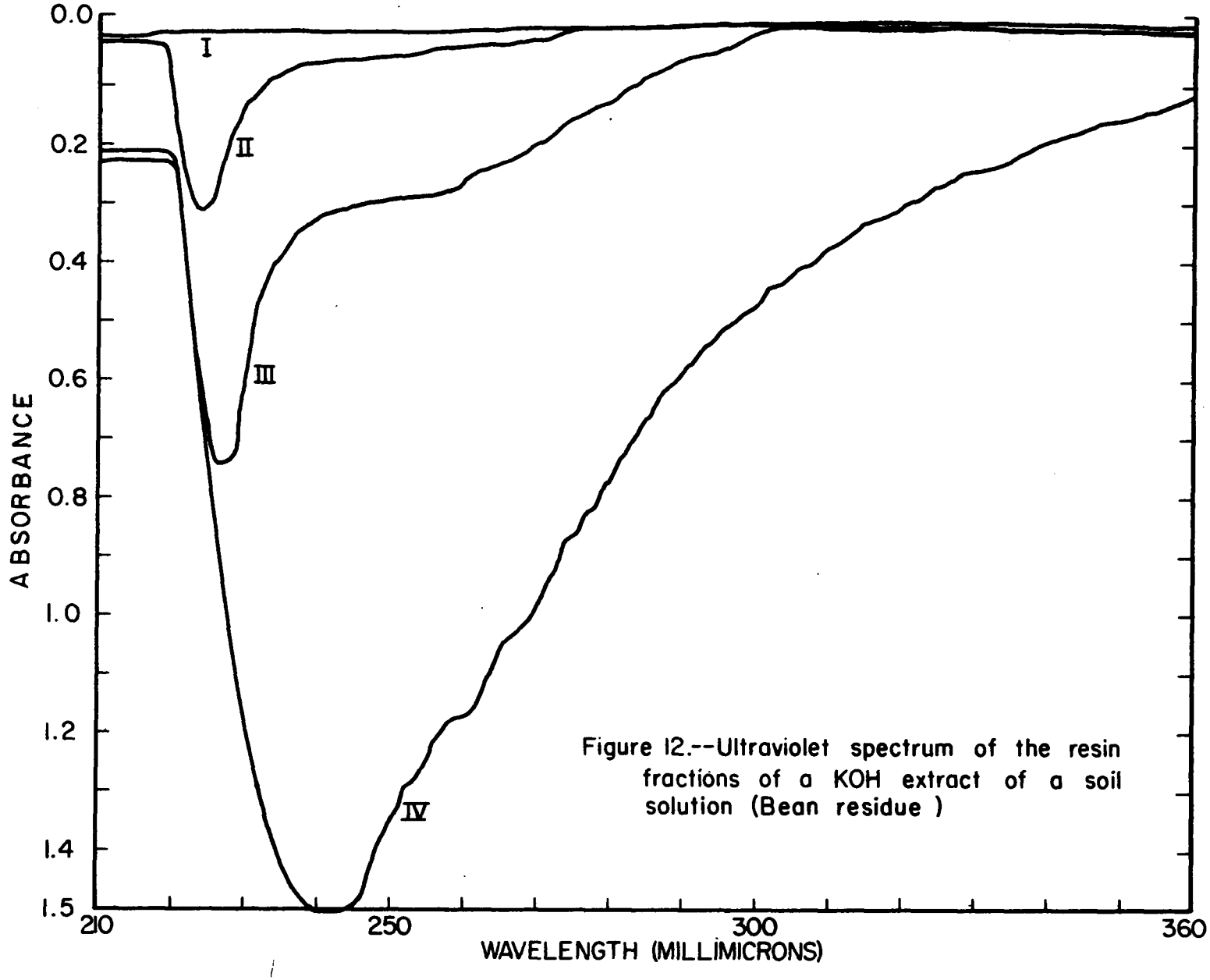

Figure 12.--Ultraviolet spectrum of the resin fractions of a KOH extract of a soil solution (Bean residue)

fractions II and III as compared to fraction IV. Such results point to phosphorus of fraction IV as a minimum estimation for RNA and nucleotide-phosphorus.

The presence of RNA and related compounds in the soil solution in relatively high concentration is not surprising since Bartholomew and Goring (8) have shown that RNA phosphorus represented more than 50 percent of cell phosphorus of Aerobacter aerogenes (a soil bacterium) when the cultures were incubated with 1 percent betonite and represented 35 percent of the cell phosphorus in case of pure cultures.

General Discussion

In the light of the presented physical and chemical fractionation of soil solution, it appeared that phosphorus moved downward in the soil columns with the soil solution in two main forms: organic and inorganic phosphorus. Treatments that accelerated the phosphorus movement, namely sucrose + NH_4NO_3 and bean residue, increased the mobile organic phosphorus several fold and left water soluble inorganic phosphorus relatively unchanged. The ratio of organic to inorganic phosphorus appearing in the solution of non-treated soils was found to depend mainly on the organic matter content of the soil and the percent of calcium carbonate in the soil. The higher the calcium carbonate content of the soil, the lower will be the expected mobility of inorganic phosphorus and the higher the organic matter content, the higher will be the expected mobility of organic phosphorus.

The main part of organic phosphates moving with soil solution appeared to be physically associated with particles and most probably present within microbial cells and debris. Such a physical disposition was thought to be the main reason for the unavailability to plants of organic phosphorus moving with the soil solution. Chemically, organic phosphate appeared to be organic phosphorus compounds of biological interest, which are necessarily present in living cells, such as RNA, nucleotides, phospholipids, phosphoproteins and phosphate esters of intermediate metabolism. From these compounds RNA nucleotide-phosphorus and related compounds were detected in relatively large amounts in the displaced soil solutions. Phosphates extracted with lipid solvents were present in small amounts. Small amounts of phosphates behaved similar to glucose monophosphates, glycerophosphates and other monophosphate esters of low molecular weight. A considerable amount of phosphorus appeared in the acid soluble fraction and behaved like phytate phosphorus, but attempts to separate phytate phosphorus as a ferric salt did not succeed. It is thought that phytin was probably present but in an amount smaller than that required for successful precipitation as a ferric salt. The presence of phytin and phytin-related compounds in the soil solutions that were displaced from sucrose-treated columns indicates the probable role of soil microflora in producing inositol phosphates. DNA was assumed to be present in very small amounts if present at all since fraction I of the resin columns

did not show any significant absorption in the ultraviolet region. Phosphoproteins probably accounted for a small amount of mobile phosphorus but no trials were undertaken to evaluate their presence.

In very broad terms it appears that the principal and most effective means for transporting phosphorus along with the soil solution is the soil microorganisms and whatever phosphate compounds are present in the microbial cells will consequently appear in the soil solution.

SUMMARY

An investigation was undertaken to characterize the phosphorus compounds moving downward in a calcareous soil. Soil columns, filled with soil material taken from the B horizon of Mohave sandy loam, were treated in duplicates with sucrose + NH_4NO_3, bean residue and no treatment. Phosphorus compounds moving with the soil solutions of these columns were studied both with respect to their physical disposition and their chemical nature. Millipore filtration and anion exchange chromatography were the principle techniques employed in this study.

The displacement of soil solution of the untreated columns with deionized water induced little phosphorus movement with more than one-half of the mobile phosphorus being organic. The application of organic matter extensively increased the movement of organic phosphorus and left inorganic phosphorus relatively unchanged.

More than 90 percent of total organic phosphorus moving with the soil solution was particulate in nature and only 10 percent or less was actually water soluble. The major portion of the particulate phosphorus was associated with particles that were larger in size than 0.45μ. Phosphorus carrying particles, smaller than 0.45μ, accounted for only 20 percent of total phosphorus movement.

Microbial cells and debris appeared to be the predominant phosphorus carrying particles in the soil solutions. It is probably because of their being sequestered within microbial cells that the mobile soil-organic-phosphates are not available to plants.

More than 80 percent of mobile soil phosphorus was base soluble. Potassium hydroxide extracts were found to contain fairly large amounts of organic phosphorus that resembled in behavior RNA and nucleotide-phosphorus. The ultra violet absorption spectra of the KOH-extracts substantiated the presence of RNA and RNA related compounds in KOH-extracts of the soil solution.

Small amounts of mobile phosphorus were extracted with lipid solvents following the base extraction and were considered phospholipids. Small amounts of the base soluble phosphate esters behaved in a manner similar to that of glucose phosphates and glycerophosphates.

A large portion of mobile soil phosphorus was associated with the acid soluble fraction which probably contained a considerable amount of phytate-phosphorus and other related phosphorus compounds. The presence of phytate-phosphorus in the soil solutions of sucrose treated columns, suggests that soil phytin is probably a microbial product.

It was concluded that microbial cells and debris are the principal means for transporting phosphorus through the soil profile and whatever are the phosphate compounds present within the cells will

consequently appear in the displaced soil solution and will account for a large portion of mobile phosphorus.

APPENDIX

Table 7. Distribution of Phosphorus in the Size Fractions of Displacement No. 1, as Separated by Millipore Filtration.

Treatment		Phosphorus						
		Total	Water soluble inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ) Total	"F" (<10mμ) Organic
		μg/displacement						
None	1	35	16	19	12	3*	20	4
	2	38	14	24	15	3*	18	4
Sucrose + NH_4NO_3	1	95	20	75	46	23	28	8
	2	87	24	63	35	22	30	6
Bean residue	1	150	22	128	85	25	43	21
	2	155	20	135	96	30	28	8

*Calculated values

Table 8. Distribution of Phosphorus in the Size Fractions of Displacement No. 2, as Separated by Millipore Filtration.

Treatment		Phosphorus						
		Total	Water soluble inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ) Total	"F" (<10mμ) Organic
		μg/displacement						
None	1	35	15	20	20	--*	15	--
	2	45	14	31	25	5*	15	1
Sucrose + NH_4NO_3	1	360	20	340	240	53	55	35
	2	430	18	412	334	45	40	22
Bean residue	1	310	22	288	187	50	57	35
	2	235	16	219	137	37	55	39

*Calculated values

Table 9. Distribution of Phosphorus in the Size Fractions of Displacement No. 3, as Separated by Millipore Filtration.

Treatment		Phosphorus						
		Total	Water soluble inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ) Total	"F" (<10mμ) Organic
		μg/displacement						
None	1	28	16	12	11	--*	18	2
	2	30	16	14	11	--*	19	3
Sucrose + NH_4NO_3	1	335	22	313	253	38	35	13
	2	360	26	334	273	42	35	9
Bean residue	1	190	24	166	127	25	30	6
	2	180	22	158	120	22	30	8

*Calculated values

Table 10. Distribution of Phosphorus in the Size Fractions of Displacement No. 4, as Separated by Millipore Filtration.

Treatment		Phosphorus						
		Total	Water soluble Inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ) Total	"F" (<10mμ) Organic
		μg/displacement						
None	1	37	18	19	20	--*	18	--
	2	45	17	28	25	2*	18	1
Sucrose + NH_4NO_3	1	300	24	276	200	50	35	11
	2	260	20	240	146	55	40	20
Bean residue	1	175	22	153	104	40	25	3
	2	150	20	130	93	30	22	2

*Calculated values

Table 11. Distribution of Phosphorus in the Size Fractions of Displacement No. 5, as Separated by Millipore Filtration.

Treatment		Phosphorus						
		Total	Water soluble inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ)	
							Total	Organic
		μg/displacement						
None	1	35	20	15	15	--*	20	--
	2	38	18	20	17	1*	20	2
Sucrose + NH_4NO_3	1	255	24	231	180	35	30	6
	2	220	20	200	163	25	25	5
Bean residue	1	145	26	119	100	15*	30	4
	2	120	22	98	83	12*	25	3

*Calculated values

Table 12. Distribution of Phosphorus in the Size Fractions of Displacement No. 6, as Separated by Millipore Filtration.

Treatment		Phosphorus						
		Total	Water soluble inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ) Total	"F" (<10mμ) Organic
		μg/displacement						
None	1	45	18	27	25	--*	20	2
	2	40	19	21	17	3*	20	1
Sucrose + NH_4NO_3	1	260	24	236	193	25	35	11
	2	220	18	202	170	20	25	7
Bean residue	1	120	18	102	70	23	23	5
	2	130	18	112	83	20	20	2

*Calculated values

Table 13. Distribution of Phosphorus in the Size Fractions of a Total of Six Displacements.

Treatment		Phosphorus						
		Total	Water soluble inorganic	Organic	"R_1" (>0.45μ)	"R_2" (0.45μ to 10mμ)	"F" (<10mμ)	
							Total	Organic
		μg						
None	1	215	103	112	103	3	111	8
	2	236	98	138	110	13	110	14
Sucrose + NH_4NO_3	1	1605	134	1471	1112	224	218	84
	2	1577	126	1451	1121	209	195	69
Bean residue	1	1090	134	956	673	178	208	74
	2	970	118	852	612	151	180	63

Table 14. Distribution of Phosphorus in the Chemical Fractions of Displacement No. 1.

Treatment		Phosphorus							
		Total	KOH-Insoluble	KOH-Soluble	In the resin fractions				
					I	II		III	IV
						Total	Inorganic		
		µg/displacement							
None	1	35	5	30	-	19	18	6	6
	2	38	4	34	-	20	18	6	6
Sucrose + NH_4NO_3	1	95	12	80	6	38	32	16	17
	2	87	10	75	6	41	34	12	15
Bean residue	1	150	28	118	4	41	30	32	37
	2	155	26	130	4	43	32	35	43

Table 15. Distribution of Phosphorus in the Chemical Fractions of Displacement No. 2.

Treatment		Phosphorus							
					In the resin fractions				
		Total	KOH-Insoluble	KOH-Soluble	I	II		III	IV
						Total	Inorganic		
		µg/displacement							
None	1	35	5	30	-	22	21	-	6
	2	45	6	40	4	19	18	6	10
Sucrose + NH_4NO_3	1	360	52	300	22	67	42	93	106
	2	430	68	350	25	81	47	125	106
Bean residue	1	310	42	260	26	70	51	58	87
	2	235	32	200	19	84	48	32	54

Table 16. Distribution of Phosphorus in the Chemical Fractions of Displacement No. 3.

Treatment		Phosphorus							
					In the resin fractions				
		Total	KOH-Insoluble	KOH-Soluble	I	II		III	IV
						Total	Inorganic		
		µg/displacement							
None	1	28	4	23	-	14	12	4	6
	2	30	5	25	-	15	15	5	6
Sucrose + NH_4NO_3	1	355	57	290	12	54	40	92	120
	2	360	63	290	13	52	42	80	128
Bean residue	1	190	32	155	9	48	32	42	46
	2	180	26	150	7	60	38	32	44

Table 17. Distribution of Phosphorus in the Chemical Fractions of Displacement No. 4.

Treatment		Phosphorus							
					In the resin fractions				
		Total	KOH-Insoluble	KOH-Soluble	I	II		III	IV
						Total	Inorganic		
		µg/displacement							
None	1	37	6	30	-	22	20	-	5
	2	45	8	36	-	30	30	-	5
Sucrose + NH_4NO_3	1	300	48	244	25	58	40	65	86
	2	260	40	218	18	54	40	68	82
Bean residue	1	175	34	138	15	46	36	34	38
	2	150	26	120	12	44	32	24	32

Table 18. Distribution of Phosphorus in the Chemical Fractions of Displacement No. 5.

Treatment		Phosphorus							
					In the resin fractions				
		Total	KOH-Insoluble	KOH-Soluble	I	II		III	IV
						Total	Inorganic		
		μg/displacement							
None	1	35	5	30	-	22	22	4	4
	2	38	5	32	-	21	20	4	5
Sucrose + NH_4NO_3	1	255	59	185	20	52	42	52	45
	2	220	45	170	16	54	38	35	58
Bean residue	1	145	26	115	12	48	36	21	28
	2	120	19	98	14	42	32	15	24

Table 19. Distribution of Phosphorus in the Chemical Fractions of Displacement No. 6.

Treatment		Phosphorus							
					In the resin fractions				
		Total	KOH-Insoluble	KOH-Soluble	I	II		III	IV
						Total	Inorganic		
		μg/displacement							
None	1	45	6	38	4	22	20	5	6
	2	40	5	335	4	22	20	5	5
Sucrose + NH_4NO_3	1	260	58	196	18	58	40	52	62
	2	220	42	172	15	50	36	44	57
Bean residue	1	120	22	94	12	39	28	18	22
	2	130	20	105	10	44	30	21	28

Table 20. Distribution of Phosphorus in the Chemical Fractions of a Total of Six Displacements.

Treatment		Phosphorus							
		Total	KOH-Insolbule	KOH-Soluble	In the resin fractions				
					I	II		III	IV
						Total	Inorganic		
		μg							
None	1	215	31	181	4	121	113	19	23
	2	236	33	202	8	127	121	26	37
Sucrose + NH_4NO_3	1	1605	286	1295	103	327	236	370	437
	2	1577	268	1275	93	332	237	364	446
Bean residue	1	1090	184	880	80	292	213	205	258
	2	970	149	803	68	317	212	159	225

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