

STUDY OF THE EFFECT OF SUCCESSIVE CROPPINGS
ON ORGANIC PHOSPHORUS CONTENT OF A SELECTED
GHANAIAN SOIL USING FOUR DIFFERENT CROPS

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I N T R O D U C T I O N

Phosphorus does occur both in organic and inorganic forms, and a relatively large amount is present in organic form. Relatively little work has been done on organic phosphorus in Ghanaian soils. Some research workers notably Schollemberger (1920), Dean (1938) and a number of others have found that soils high in organic matter are also high in organic phosphorus. Nye and Bertheux (1957) working on Ghanaian soils observed considerably more organic phosphorus in forest soils than in savanna soils.

There is much controversy on the form in which phosphorus is taken up by the plant. It is a general belief that organic phosphorus is utilised by the plant only when it is changed into inorganic orthophosphate form. When organic matter decomposes, the free orthophosphate ions released into the soil solution are either taken up by the plant or combine with other soil constituents. Therefore the organic phosphorus serves as a reserve upon which the plant falls when the inorganic phosphorus present in the soil solution is depleted. However, there is the opinion that the organic phosphorus in the soil can be directly utilised by plants without it being converted first into inorganic form and some workers have tried to establish this fact. Bottomley (1919), Heck and Whiting (1927) and many others conducted numerous studies on the availability of different organic phosphorus compounds to determine whether the compounds were directly

absorbed by plants. Roger et al (1940) found that both phytin and lecithin were absorbed directly from nutrient solutions by corn and tomato plants. Pierre and Parker (1927) however, failed to find evidence of absorption of water soluble organic phosphorus of soils by corn, soybeans or buckwheat.

Since soil organic phosphorus can be used either directly or indirectly by the plant, it is therefore reasonable to assume that organic phosphorus status in the soil will decrease to a large extent over a long cropping period. Hayashi and Takiyama (1955) observed the decrease in organic phosphorus content of the soil by growing plants. This observation was later verified by Sekhon and Black (1969) and Thompson and Black (1970). However, Thompson (1967) found that corn and tomato plants decreased organic phosphorus in short period, whilst these same plants increased the organic phosphorus over a long growing period. As different crops have different absorbing capacities for phosphorus, the degree of decrease or increase of organic phosphorus through cropping should vary from crop to crop.

It has long been suspected that the decrease in organic phosphorus content of the soil in the presence of plant is induced by enzyme phosphatase secreted by the plant, and this enzyme converts the organic phosphorus into the inorganic form. Investigations therefore have been conducted into this 'phosphatase activity' hypothesis. Thompson

and Black (1970) verified this hypothesis and obtained positive results. Thus there are two reasonable possibilities which may cause the decrease.

They are:

- (a) An increased phosphatase activity in the soil ('Phosphatase Activity' Hypothesis).
- (b) The Direct absorption of organic phosphorus by plants ('Direct Absorption' Hypothesis').

Over a long period, a large amount of work has been carried out on the organic matter content of temperate soils, while in the tropics, the question of the level of organic matter during cropping has scarcely been touched. The general belief is that when the native vegetative cover of soils is destroyed, there is a rapid decay of the organic matter that has accumulated in them over a long period of time. This stimulated decomposition usually results in a rather rapid decrease in the organic matter content of soils when they are cropped. Shutt (1925) found that a prairie soil lost about 25% of its humus content in twenty-two years of cropping. Blair and Prince (1936) in a study of the soils on nineteen farms in a county observed an average of 2.71% of humus in the forest soils and 1.84% in the corresponding cropped soils. However, Coutler (1950) observed that heavily cropped soils do not lose all their organic matter, and there

is even a slow build up of organic matter under cropping.

The objectives of this thesis therefore are:

- (1) To verify the observation that plants in fact decrease the organic phosphorus content of soil, and the extent of the decrease by different crops through successive croppings.
- (2) To find out whether the decrease of organic phosphorus, if any, is the result of an enhanced phosphatase activity in the mineralization of the organic phosphorus into inorganic phosphorus which is finally absorbed by the plant or the result of direct absorption of organic phosphorus by plants.
- (3) To find out if there is any change in the organic matter content of soil during the successive cropping periods.

CHAPTER 1

LITERATURE REVIEW

THE ROLE OF SOIL ORGANIC PHOSPHORUS IN PLANT NUTRITION

Conflicting views are held by many investigators as regards the significance of soil organic phosphorus in the phosphorus nutrition of plants. The results of Pierre and Parker's (1926) experiments showed that the organic phosphorus in the displaced solution or in the soil extract was not directly available to plants. Bower (1949) also noted that soil organic phosphorus may undergo mineralization, and Thompson and Black (1949) also observed a relationship between the amounts of organic phosphorus, nitrogen and carbon mineralized during soil organic matter decomposition. Thompson and Black (1949) strongly believed that (from their experimental results) soil organic phosphorus should also be of importance in plant nutrition, using analogy with soil organic nitrogen. The analogy is that since nitrogen and phosphorus of the organic matter in soils exist in a definite ratio, the process by which organic nitrogen is mineralized should be similar to that of organic phosphorus mineralization. In a review of the phosphorus cycle, Pierre (1948) emphasized the probable importance of soil organic phosphorus in plant nutrition.

The unavailability of the organic phosphorus directly to plants

does not mean that such phosphorus may not be made available to the plant by biological agencies within the soil. It is probable that in soils, organic phosphorus is broken down and the inorganic phosphorus is made available to the plants as a result of microbial action. Pierre and Parker (1926) showed that water-soluble organic phosphate is not absorbed by plants and is consequently unavailable to the plant as long as it remains in the organic form.

Since the residues of higher plants and microorganisms are the principal sources of organic phosphorus in soils, it is to be expected that nucleic acid, phytin and lecithin and their intermediate decomposition products constitute the bulk of the organic phosphorus found in soils. This assumption is supported by several workers (Bottomley (1919), Shorey (1912), Wrenshall et al (1940), Yoshida (1940) who have shown the presence of nucleic acids, nucleotides and phytin in relatively large amounts and lecithin in small quantities in soils. Adams et al (1953) have suggested that nucleic acids comprise only about 5% of the soil organic phosphorus. Wrenshall and McKibbin (1937) also calculated that 47.5 to 65% of the soil organic phosphorus was nucleic acids, whilst Bower (1945) found the amount to range from 17 to 33%. Hance and Anderson (1962) obtained values for phospholipids extracted from five soils ranging from 0.31 to 0.70 mgP/100 gm of soil and these accounted for 0.6 to 0.9% of the total organic phosphorus.

Bower (1945) also found that about 1% of the total soil phosphorus is lecithin.

Evidence has been obtained that both lecithin and phytin can serve as phosphorus-sources for higher plants even under sterile conditions, ~~but~~ phytin producing a greater effect than lecithin. Wiessflog and Mengdehl (1933) found that, under aseptic conditions, glycerolphosphate, sugar phosphates, inositol hexaphosphate and nucleic acid provided good sources of phosphate to maize which gave yields at least as high as those from inorganic phosphate. Szember (1960) has confirmed the availability of inositol hexaphosphate to plants under aseptic conditions. Wild and Oke (1965), in their studies of organic phosphate compounds in CaCl_2 extracts of soils, found that one form of inositol phosphate was resistant to hydrolysis and had a low availability to plants grown under aseptic conditions. The other was more easily hydrolysed and was readily used by plants. Bremner (1951) has also suggested that organic phosphorus could be absorbed directly by plants to some extent.

It has been found by many workers notably Rogers *et al.* (1940), Betramson and Stephenson (1942), Bower (1949) and Conrad (1939) that in solution and sand cultures, the phosphorus added as inositol hexaphosphates (or phytin) is readily available to plants, but that in soils especially those of low pH values, the availability is greatly

reduced. Bower (1949), observed that the availability of the phosphorus of the lower inositol phosphates (or phytin derivatives) is similarly related to the soil pH but is greater than that of the hexaphosphate. Jackman and Black (1951), in their studies of the solubilities of Iron, Aluminium, Calcium and Magnesium salts of inositol hexaphosphoric acid (phytic acid) and of mixture of lower inositol phosphoric acids having an average of 2.71 atoms per molecule, concluded that insofar as solubility determines the availability of phosphorus in soil inositol phosphates, agronomic practices designed to maintain the soil reaction at about pH 6.0 are desirable.

THE EFFECT OF PLANTS ON SOIL ORGANIC PHOSPHORUS

Some investigators hold the view that growth of plants decreases the organic phosphorus content of soils. Vincent (1937), Williams (1950) and Hayashi and Takijima (1955) have made measurements of organic phosphorus mineralization in experiments relating directly to utilization of soil organic phosphorus by plants. Vincent (1937) found that the amount of soil organic phosphorus extracted by dilute citric acid solution decreased during the growth of a crop on the soil. Williams (1950) also noted that the organic phosphorus soluble in diluted NaOH did decrease but not appreciably, during the growth of a crop on soil. Hayashi and Takijima (1955) also observed that the total organic phosphorus content of soil decreased during the

growth of a crop.

Some research workers believe that the decrease in soil organic phosphorus is concentrated in a thin layer of soil around the roots. To investigate this possibility, Sekhom and Black (1969) made organic phosphorus determinations on soil aggregates that adhered to the separated roots and compared these values with organic phosphorus determined in the bulk soil. They found no significant differences between the bulk soil and the aggregates adhering to the roots. The experiments conducted by Thompson and Black (1970) were done to test further the presumed effect of plants on soil organic phosphorus, with emphasis on the soil in the rhizosphere. They observed that plants contributed organic phosphorus to the soil, but did not affect the original soil organic phosphorus significantly. As a whole, the results of the experiments under artificial conditions verified the previous findings that soil organic phosphorus may decrease as a result of the activity of growing plants. The results showed, in addition, that a plant-induced decrease in soil organic phosphorus may occur when roots and soil are physically segregated and not in direct contact. This effect was presumably a consequence of transference of substances in solution between soil solids and roots. Thompson (1967) investigated the short term effects of plants on the soil organic phosphorus content of soil using corn and tomato seedlings.

In two of three experiments with each of the two crops, the loss of organic phosphorus in the presence of a plant exceeded the loss of organic phosphorus in the absence of a plant. In one experiment with each crop, however, the loss of organic phosphorus in the control was greater than the loss from the soil in the presence of plant. The variability of the results was greater with corn than with tomatoes. This behaviour was explained by Thompson (1967) on the basis that the presence of corn plants caused addition of organic phosphorus to the external medium either directly or indirectly through release of organic substances that caused an increase in numbers of microorganisms and hence in production of organic phosphorus.

AMOUNTS AND DISTRIBUTION OF ORGANIC PHOSPHORUS IN SOILS

Considerable knowledge has been accumulated on the amounts of organic phosphorus present in soils. Schollenberger's (1918) results agreed with those of Potter and Benton (1916) in showing that there is a rather large amount of soil phosphorus in the organic form. In his study of organic phosphorus content of Ohio soils, Schollenberger (1920) found that one-third and one-fifth of total phosphorus present in the surface and subsurface samples respectively of both virgin and cultivated soils were in organic form. Fuller and McGeorge (1950) also observed that about one-third of the total phosphorus of Calcareous Arizona soils was organic.

Acquaye (1962) using forest soils, Antoakrom, Domlabra, Brofoyedru and Obuasi series from Ghana, found that the organic phosphorus content ranged from 46.0 to 69.5% of the total phosphorus. Enwezor and Moore (1966), working with Nigerian soils, also noted that organic phosphorus constituted 43 and 28% of the total phosphorus in the 0-6" and 6-12" layers of forest soils and 18 and 10% in the corresponding layers of savanna soils. Nye and Bertheux (1957) observed that there is considerably more organic phosphorus in the forest than the savanna soils of Ghana and that the amount fell with depth, but it fell more slowly than might be expected from the fall in total organic matter. John et al (1965) examined surface horizons of 38 British Columbian soils belonging to six different orders and found that the concentration of organic phosphorus was 1.8 to 77.7% of the total phosphorus. They also observed that the wide range of organic phosphorus content with some soil orders showed that its distribution was not entirely characteristic of the soil order.

Some workers have found a close relationship between the organic phosphorus, organic carbon and nitrogen contents in a soil. Using direct method of analysis, Schollenberger (1920) found that the quantities of organic phosphorus, nitrogen and ammonium hydroxide-soluble organic matter were correlated in twenty four

samples of surface soil horizons from Ohio, the average ratio of nitrogen to organic phosphorus being 10.0. Dean (1938) observed that the amount of organic phosphorus in the soil was closely related to the carbon content. Pearson and Simonson (1940) determined the vertical distribution of organic phosphorus, nitrogen and carbon in seven Iowa soil profiles and found a definite correlation among the quantities of the three components present. The average ratio of nitrogen to organic phosphorus was 11.2 and the average ratio of organic carbon to organic phosphorus was 116; for the surface horizons, the corresponding ratios were 8.8 and 109. Black and Goring (1953) also have pointed out that, positive correlation exists between the content of organic phosphorus and the contents of organic carbon and nitrogen in soils. Nye and Bertheux (1957) also found a close correlation between the organic phosphorus and the organic carbon content. Acquaye (1962) also noted that the organic phosphorus content was correlated to the percentage total nitrogen, total organic carbon and pH.

FACTORS AFFECTING THE CONTENT AND COMPOSITION OF SOIL ORGANIC PHOSPHORUS

Numerous workers have conducted a number of investigations into the factors which affect the content and chemical composition of organic phosphorus in soils. Walker and Adams (1959) determined the chemical composition of the organic matter of soils formed on

similar parent material, but under a range of climatic conditions. Their results showed that, as rainfall increased, the phosphorus content of the organic matter tended to be lower.

Temperature has been known to affect the content of organic phosphorus of soil. Van der marel (1947) found mineralization of organic phosphorus of soils to be rapid under tropical conditions. The results of Walker and Adams (1959) experiments also indicated that the phosphorus content of the organic matter of soil tended to be lower when the mean temperature was higher. The mineralization of soil organic phosphorus takes place very rapidly when moist soil is also heated. Bower (1949) reported that when soils are incubated under favourable conditions, some of the organic phosphorus is changed to the inorganic form. He measured the mineralization by determining the increase in acid-soluble phosphorus produced in the soil during incubation, and checked his results in a few soils by measuring the decrease in organic phosphorus. Ghani and Aleem (1943), and Thompson and Black (1949) also observed that substantial mineralization of soil organic phosphorus may occur when soils high in organic phosphorus are incubated under conditions of moisture and temperature favourable to plant growth. Thompson and Black (1947) incubated three Iowa soils for one week at temperatures ranging from -14°C to 150°C and the increase in inorganic phosphorus soluble in $1\text{N H}_2\text{SO}_4$ was

determined as a measure of the mineralization of phosphorus originally contained in organic form. They observed that the increase in acid-soluble phosphorus over the temperature range from -14°C to 30°C did not differ significantly (average 50 ppm), but above 30°C , there was a very rapid increase continuing up to 150°C at which temperature the soil organic phosphorus was completely mineralized. They also reported that the increase in acid-soluble at higher temperatures was due in part, to increased solubility of the inorganic phosphorus of the soil. From their work, it seems that there is a possibility of the ratio of nitrogen to phosphorus decreasing with increasing soil temperature. However, the incubation tests conducted by Nye and Bertheux (1957) revealed little mineralization of the soil organic phosphorus fraction.

It is believed that pH is a major factor in determining the proportion of the total phosphorus that might exist in organic form. This knowledge is of particular importance because it leads to the suggestion that raising the pH of acid soils might "unlock the store house" of phosphorus tied up in organic matter. There are some reports that the phosphorus content of organic matter is lower in alkaline than in acid soils. Williams and Steinbergs (1958) found that the phosphorus content of organic matter tended to be lower when the pH was above 7. Williams, Williams and Scott (1960) found that

the phosphorus content of the organic matter was lower in calcareous soils. On the other hand, Walker and Adams (1958) observed that for the one alkaline soil they investigated, the phosphorus content of the organic matter was higher than the average of other soils. Karim and Khan (1955) working on some East Pakistan soils found that as the pH rose from 4.2 to 4.6, the organic phosphorus increased, with further rise of pH from 4.6 to 5.6, this form decreased, but increased again with further rise of pH, that is above pH 5.6. The results of Thompson et al (1954) also indicated that the soil pH is a modifying influence on soil organic phosphorus mineralization. Their results obtained with the group of 50 soil samples confirmed laboratory observations of Ghani and Aleem (1943), Goring and Bartholomew (1951; 52) to the effect that mineralization of soil organic phosphorus increases with increasing pH. They also observed that the organic phosphorus mineralization increased with soil pH not only during laboratory incubation; but also during soil development. However, Moore (1959) determined mineralization of soil organic phosphorus by extraction with 0.2N HCl (1:50 soil - solution ratio) before and after partially anaerobic and wholly aerobic incubation for 20-40 days for two sandy loam soils of pH 7.9 and 5.9. He found that while mineralization of phosphorus under anaerobic conditions was almost identical in both soils, considerably greater amounts of

mineralized phosphorus were found after aerobic incubation in the soil of pH 5.9. Schollenberger (1920) observed, however that, the reaction of the soil appeared to have no influence upon the quantity and nature of organic phosphorus present.

There are two difficulties when interpreting such reports that alkaline soils have lower organic phosphorus content than acid soils. One of the difficulties is that extraction methods may give low values for organic phosphorus in alkaline soils. This difficulty applied to the work of Williams et al (1960). The other is that alkaline soils have often been formed under different climatic conditions from acid soils, and the effects of alkalinity are therefore confounded with climate.

The length of time for which the climate has operated on a soil probably affects the phosphorus content of the organic matter. In a study of development of soil from loess, Godfrey and Riecken (1954) found that as the profile developed, the organic phosphorus of the soil tended to fall. In a similar study on alluvium, Geel and Agarwal (1959) found that the organic phosphorus contents of soils were only 10 to 20% of the total phosphorus in all the soils which decrease with maturity of the soils.

There are other possible factors which may also affect the organic phosphorus in soils, but the effect may not be very pronounced.

Williams and Saunders (1956) showed that poorly drained soils contained less organic phosphorus than well drained ones, and in a paper by Williams et al (1960), it was stated that the C/P ratio was much lower when drainage was poor. The effects of parent material have also been considered by Williams et al (1960) and William and Steinberg (1958). Small effects were observed; for example, soil derived from granite tended to have a lower phosphorus content in the organic matter than soils derived from basalt or basic igneous material.

METHODS OF DETERMINATION OF ORGANIC PHOSPHORUS

Methods for the determination of organic phosphorus can conveniently be grouped under two main headings:

- (a) "Extraction" Methods
- (b) "Ignition" Methods

"Extraction" Methods

These methods involve the extraction of soils with dilute acids and alkalis, and the total and inorganic phosphorus of the extract determined. The difference between the total and inorganic phosphorus is taken to be the amount of organic phosphorus present in the soil. Potter and Benton (1916) first attempted to determine the organic phosphorus in soil and they devised a method which proved quite satisfactory. Schollenberger (1918) studied the problem later and

modified the method slightly. A procedure was adopted by Pearson (1940) in which the organic compounds of phosphorus are removed from the soil by acid extraction followed by digestion in dilute ammonium hydroxide. Organic phosphorus is found by difference between inorganic and total phosphorus in the extracts. Mehta et al (1954) also developed a method which involves, pretreating the soil with concentrated HCl, and extracting the same soil with .5N NaOH at room temperature and with .5N NaOH at 90°C for 8 hours. MacLean (1965) reported that the method of Mehta et al (1954) was effective in estimating the total organic phosphorus content of podzols, but felt that the method involved rather drastic treatment of the soils. Anderson (1960) stated that some hydrolysis of organic phosphorus compounds, including inositol hexaphosphate, occurred when the method of Mehta et al (1954) was used. Diest and Black (1958) compared the methods of Kaila and Virtanen (1955) and Mehta et al (1954) and their results substantiated the findings of Kaila and Virtanen (1955) that amounts of organic phosphorus found in different soils by the two methods are not related to each other in a strictly proportional manner.

Harrap (1963) used Sodium EDTA to extract organic phosphorus from soils, and suggested that the $\text{Na}_2\text{EDTA}/\text{NaOH}$ method was satisfactory for determining soil organic phosphorus, except on very acid soils, where the Mehta et al (1954) method could be used. MacLean (1965)

proposed another method which involves extracting the soil with 0.5M NaHCO_3 at pH 10.0 for 16 hours at 90°C following acid pretreatment with 0.5N HCl for 1 hour. Dormaar (1968) compared an alkaline extraction consisting of pretreatment with 0.1N HCl and extraction by 1N NaOH followed by acidification with H_2SO_4 at pH 1.0 with a chelating resin extraction consisting of a 0.1N HCl-0.1N HF pretreatment and a 15 hour Dowex A-1 (Na^+) resin extraction followed by removal of Na^+ via Dowex 50W-K4 (H^+) column. He observed that more of the organic phosphorus was extracted by the resin method than alkali method.

Ignition Method

This is a simple method proposed by Saunders and Williams (1955) which involves igniting air dried soil for 1 hour at 550°C and extracting the ignited soil and comparable sample of unignited soil with 0.2N H_2SO_4 for 16 hours. Inorganic phosphorus is determined colorimetrically in both samples and the difference taken as organic phosphorus.

Walker and Adams (1958) observed that the highest value for organic phosphorus was obtained with 1N H_2SO_4 as the extractant. Legg and Black (1955) used concentrated HCl in the extraction after ignition; in their work, the period of extraction was about one and half hours.

Methods of determination of organic phosphorus have been compared with each other to find which of them gives the highest values for organic phosphorus. Enwezor and Moore (1965) made a comparison

between the HCl/NaOH Method and the ignition method of Saunders and Williams (1955) and found that ignition method gave much higher organic phosphorus values than the extraction method. Legg and Black (1955) found little difference between an ignition method using a lower temperature (240°C) and the extraction method of Mehta et al (1954) applied to a range of United States soils. Saunders and Williams (1955) compared extraction and ignition methods on Scottish soils and obtained slightly higher values with ignition method. However, Hance and Anderson (1962) found that estimates by ignition and extraction methods generally show fair agreement. This result agreed with that of Saunders (1959). Williams and Saunders (1955) suggested that ignition of highly sesquioxide soils would probably give low values for organic phosphorus but this is not borne out by the data presented by Enwezor and Moore (1965).

However, there is evidence that extraction methods can also give higher values of organic phosphorus than ignition methods. Harrap (1963) using English soils, found that HCl/NaOH extraction gave higher values than an ignition method (240°C) for soils with a pH less than 5 but comparable results for soils with a pH greater than 5. The HCl/NaOH extraction method employed by Enwezor and Moore (1965) on Nigerian soils gave relatively low values for organic phosphorus but the values were similar to the average value of organic

obtained by Nye and Bertheux (1957) for 67 Savanna soils in Ghana, using an acetic acid /NaOH extraction method of Williams.

Attempts have been made to explain the high values of organic phosphorus by using either extraction or ignition method. Williams and Walker (1967) comparing the ignition and extraction methods found that ignition method markedly increased the solubility in dilute acid, of iron and aluminium-bound inorganic phosphate. This observation thus supports the view that ignition method may sometimes overestimate the total organic phosphorus in soils. Halstead and Anderson (1969) also found that extraction with hot 3N NaOH removed more total organic phosphorus, and inositol phosphorus fractions from Scottish and Canadian soils than did extraction with hot 6N HCl, hot concentrated HCl or 0.2M aqueous acetyl-acetone at pH 8.0. They also attributed this higher value to increase in solubility of the inorganic phosphate in the NaOH.

MICROORGANISMS AND SOIL ORGANIC PHOSPHORUS

With the view to obtain definite information regarding the influence of microorganisms on the uptake of phosphate by plants, Gerretsen (1947) grew oats, mustard, sunflower and grape under sterile and infected conditions in pot cultures, to which insoluble or difficulty soluble phosphates were added. He observed that although

microorganisms definitely made available a certain amount of phosphorus, otherwise inaccessible to plants, there was no other favourable bacteriological activity. Attention, therefore, has been focussed on microorganisms which can attack organic phosphorus compounds and release phosphorus in forms, principally inorganic phosphate, which may be available for plant nutrition. These more practical aspects of this ability of soil microorganisms are demonstrated by the use of 'phosphobacterin', a preparation of Bacillus megatherium var. phosphaticum, in Soviet Union by Cooper (1959). Szember (1960) carried out an experiment on the decomposition of organic phosphates by soil microorganisms. According to Heck (1935), the availability and utilization of the organic phosphorus by crops must depend to a large extent upon microbiological activities.

An extensive literature has accumulated on the assimilation of phosphorus compounds by soil microorganisms, particularly the bacteria and fungi. Thompson and associates (1931) held the belief that Azotobacter chroococcum assimilated more phosphorus during the first 45 days of growth than was made available from tricalcium phosphate, but that, the reverse was true during the second period of 45 days. Chang (1939) observed that Penicillium sp. synthesised as much as 6% organic phosphorus in a 1.5% phosphate solution, whereas the mycellium of Rhizopus nigricans, Trichoderma sp. and Aspergillus sp.

contained 3-4% organic phosphorus with glucose as a source of energy. The organic phosphorus content of Aspergillus niger was slightly greater in the solution containing 1.5% phosphate than in the one containing 0.5%.

PHOSPHATASE ACTIVITY

A lot of evidence has accumulated on the phosphatase activity of both soils and plant roots. Keilling (1964), in a report on the phosphatase activity of soils, stated that about half of the soils of France exhibit a low phosphatase activity, lowest activity occurring in degraded vineyard soils and highest in soils of high organic matter content. The phosphatase activity of African soils was low, approaching that of the Sahara rather than that of the cultivated soils and forest soils in France. Panosyan and Babayan (1965), working in Soviet Union, observed that phosphatase activity of uncovered grounds of Lake Sevan was high and was determined mainly by the number of microorganisms decomposing complex proteins containing organic phosphorus.

The discovery of a vigorous phosphatase activity of corn and tomato roots by Rogers et al (1940) and the reports of Conrad (1940) of thermolabile soil catalysts capable of decomposing urea independent of microbial activity, suggests that soils may contain a variety of catalysts, one source of which may be the residues of both actively

growing and dead plant roots. Some workers do believe that microorganisms present in the soil contribute to the phosphatase activity of soils. Casida Jr. (1959) studied the phosphatase activity of some common soil fungi and found that some species of Aspergillus contain extremely active acid phosphatase for phosphorus-containing compounds similar to those found in soils, with the greatest phosphatase activity occurring at low pH, but there was some activity near neutrality. Chunderova (1964) observed that in Kharkov leached Chernozem, the phosphatase activity of particular groups of bacteria isolated from the rhizosphere was greater in maize than in millet. Galstyan and Markosyan (1965) found that in the leached Chernozem and mountain-meadow soil, the optimum pH for phosphatase was 5.4-6.0 and in brown and chestnut soils, it was 8.0-8.5.

Evidence is also available that some plants are capable of absorbing and growing on nucleic acid as phosphorus source. Ratner and Samoilova (1958) and Szember (1960) have all found that certain organic phosphorus compounds will support the growth of plants under sterile root conditions. Many investigators therefore worked on the ability of plants to utilize organic phosphorus compounds in nutrition by virtue of root enzymes. Rovira (1959) found that roots are capable of producing exudates and the nature of these exudates is influenced by temperature and light intensity variation. The activities of

invertase and phosphatase of roots have been demonstrated by Ratner and Samoilova (1958). Rogers et al (1940) were able to show a phosphatase activity of nonsterile corn roots which they attributed to enzymes of the sloughing root-cap cells. Estermann and McLaren (1961) also concluded that the phosphatase activity in the root region of plants were attributable largely to the root itself although microorganisms contribute some extra activity.

Phosphatase has been located by cytological methods in the cell wall of tomatoes by McGregor and Street (1953) and Jensen (1956) while Palade (1950) located some of this enzyme in the cytoplasm particularly the mitochondria. Estermann and McLaren (1961) also observed that the phosphatase activity was present in the cytoplasm of root cap and cortex cells, but not in nuclei or cell walls or in the stele. Hayashi and Takijima (1953) also noted that phosphatase activity particularly that of glycerophosphatase, was higher than the activity of other constituents in the roots of phosphorus-deficient cereals; and also the roots of P-deficient plants had a greater ability to mineralize organic phosphorus and absorb inorganic phosphorus. Geller et al (1960) working on Ukrainian podzolized gray soils, noted that phosphatase activity in rhizospheres of winter wheat, clover and lucerne was greater with respect to glycerophosphate than with respect to phytin and was closely related to microbial activity.

Hinde and Finch (1966) studying the activities of phosphatases, pyrophosphatases and adenosine triphosphatases from normal and boron-deficient bean roots, noted that at pH 5.1, phenyl phosphate, pyrophosphate and ATP were all hydrolysed by an acid phosphatase needing no Mg^{2+} ; at pH 7.2 and 8.2, phenylphosphate was not hydrolysed and the pyrophosphatase and ATPase acted only in the presence of Mg^{2+} . They also found that in normal roots, acid phosphatase and ATPase activities increased with distance from the tip but pyrophosphatase activity decreased.

The effect of fertilizers on phosphatase activity has been studied by Vlasyuk and Lisoval (1964) and Geller et al (1960). Vlasyuk and Lisoval (1964) found that in a 10-course rotation, mineral fertilizers increased phosphatase activity during the vegetative period in meadow-Chernozem podzolized soil. They also observed that phosphatase activity varied with time, crop and the preceeding crop. Geller et al (1960) noted that the addition of glucose and compost to the soil doubled phosphatase activity. Hoffman and Elias-Azar (1965) found that phosphatase activity was correlated to the biological availability of soil phosphorus, and may be utilized as an index where chemical determinations of phosphorus availability do not correlate with crop response.

CHAPTER II

THE EXPERIMENTAL METHODS(A) MATERIALS

Soil : The soil used in the investigation is Koforidua series sampled at 0-2" depth and from an uncultivated site of Block O at the Cocoa Research Institute, Tafo, Ghana. The soil was selected particularly for its relatively high organic matter and organic phosphorus content, so that any change in these factors that would occur during the period of the experiment could be detected. The sample was air-dried immediately after it had been collected from the field, on polythene sheets spread on drying platforms in the greenhouse for 3-5 days. The soil aggregates were crushed by pounding and were passed through 2mm sieve. The sample was bagged and stored for the experiment .

Acid-washed Sand : An imported acid-washed sand was used in the experiment. Before it was used, the sand was washed several times with distilled water and then air-dried in the greenhouse.

Crops : The crops used were -

- a) Maize (Mexican 17 variety)
- b) Tomato (local variety)
- c) Red Cowpea and
- d) Rice (SML Alupi variety)

The seeds of the first three crops were supplied by the courtesy of

Dr. S. Sinnadurai of the Department of Crop Science, University of Ghana. The rice seeds were supplied by the courtesy of Mr. William Oteng of the University of Ghana Agricultural Research Station, Kpong.

(B) GREENHOUSE EXPERIMENT

50 gm of gravel were weighed into a plastic pot which weighed 50 gm. The pot was filled with 450 gm of soil, and a rubber tube placed in the middle of the pot during filling. The pots were seeded with maize, cowpea and rice at the rate of 6, 6 and 8 seeds per pot respectively. The soil was wetted with 100 ml. of distilled water before seeding ; additional 50 ml were added after seeding. In the case of tomato, the seeds were nursed in acid-washed sand in plastic trays for two weeks. The seedlings were supplied with Hoagland solution of 1/5 strength four days after germination until they were ready for transplanting. The seedlings were then transplanted into pots at the rate of 6 seedlings per pot. Distilled water was added every morning to obtain a constant weight of 650 gm per pot. With the exception of tomato plants which were grown for 35 days, all the plants were grown for 30 days. At the end of the growth period, the plant tops were harvested. The soil was air-dried in trays for 2-4 days and the roots were sieved from the

soil, special care being exercised to remove seed residues; the soil aggregates were crushed and were made to pass through 2 mm sieve. 10 gm of the soil were taken from each pot for organic carbon and organic phosphorus determinations. Six pots were used for each crop. Controls, that is, soil with no plants were also set up. The experiment was done in duplicate.

To correct for the organic phosphorus that might be contributed by the broken roots, similar experiment was set up. In this experiment, 50 gm of gravel were weighed into a pot. The pot was filled with 450 gm of acid-washed sand that had been passed through 2mm sieve. The same number of seeds per pot used in the first set-up was used in this experiment. The seedlings were supplied with Hoagland solution of 1/5 strength, containing all the major nutrients except phosphorus, one week after germination. The tomato seedlings were however supplied with the Hoagland solution (1/5 strength) immediately after they had been transplanted into pots. Maize, cowpea and rice were grown for 30 days; the tomato plants were grown for 35 days. The plant tops were harvested and the sand was air-dried in trays. The sand was sieved through 2 mm sieve and 10 gm collected for organic carbon and organic phosphorus determinations. Controls, that is, sand with no plants were also included in the experiment. The experiment was also replicated twice. The organic phosphorus

and carbon of the sand were taken as the fractions contributed by the broken roots and thus the actual organic phosphorus or carbon content of the soil is the difference between the values of the soil and the sand. The soil and sand used previously in the first cropping period were used in the subsequent second and third cropping periods. After each growth period, the soil or the sand was sieved and organic phosphorus and organic carbon determined in 10 gm sample. The organic phosphorus and organic carbon were determined by Mehta et al (1954) and Walkley and Black (1954) methods respectively.

(c) PHOSPHATASE ACTIVITY TESTS

Maize, cowpea and rice were grown in acid-washed sand in plastic trays for seven days. In the case of tomato, the seeds were nursed for two weeks during which the seedlings were supplied with Hoagland solution of 1/5 strength. The tomato seedlings were then transplanted into pots and were allowed to grow for another two weeks. 10 seedlings each of maize, cowpea and tomato and 20 seedlings of rice were carefully uprooted from the trays and the roots washed with distilled water and finally grown in 500 ml of distilled water, and were kept in growth chamber at room temperature for one week. After the growth period, the solution was centrifuged and the supernatant was filtered through No. 42 Whatman Filter paper. The "enzyme" extracts were stored in

refrigerator for phosphatase activity tests.

1. The effect of different volumes of various 'enzyme' extracts on disodium phenylphosphate

Aliquots of 5, 10, 20, 25 ml of each extract were pipetted into 150 ml polythene shaking bottles each containing approximately 310 milligrams of phosphorus in the form of disodium phenylphosphate as organic phosphorus substrate. Appropriate quantities of distilled water were added to obtain a total volume of 50ml of solution and the bottles were incubated with shaking for 20 hours at room temperature. Blanks of 5, 10, 20, 25 ml of each extract which contained no added organic phosphorus substrate were incubated simultaneously. At the end of the incubation period, 10 ml aliquot were taken and inorganic phosphorus was determined colorimetrically on Spectronic "20" by the method of Dickman and Bray (1940) modified by Legg and Black (1955).

2. Effect of heating on extracts

To determine whether the substance responsible for any observed hydrolysis of organic phosphorus substrate in the above experiment was indeed enzyme, portions of the extracts were heated for 3 hours at 90°C in an oven. The effect of different volumes of unheated and previously heated extract on disodium phenylphosphate was then determined in the manner as described previously.

3. Effect of 'enzyme' extracts on soil organic phosphorus

1 gm samples of Koforidua soil were weighed into 100ml polythene tubes.

To one set of tubes, 1.0 ml of toluene used as a microbial inhibitor was added 30 minutes before addition of anything else. 20ml of each of the four 'enzyme' extracts were added. Each of the 4 treatments, which were soil samples with each of the 'enzyme' extracts, was made in duplicate with and without toluene. After the addition of the 'enzyme' extracts, the volume of the aqueous solutions was made up to 35 ml. with distilled water. The samples were incubated for 1 week with shaking at room temperature. The samples were then centrifuged, and the supernatant solutions were decanted. The total content of organic phosphorus in the soil plus supernatant solution was determined in the usual way.

CHAPTER III

RESULTS AND DISCUSSION

The results of the greenhouse experiments are presented in tables 1, 2, 3, 5, 6 and 7. Each value is an average of two determinations per pot after the correction for the organic phosphorus found in the sand.

Table 1 contains the mean and individual values of organic phosphorus of the original, cropped the uncropped (control) soils at the end of the 1st cropping period. The table also gives the increase or decrease in the organic phosphorus content of the cropped soils. The control and the original soil had almost the same amount of organic phosphorus at the end of the 1st cropping period. For maize, there was a decrease in organic phosphorus content in nine of the twelve pots when compared with the controls. There was however an increase in organic phosphorus content in two pots, and the other pot had almost the same organic phosphorus content as the control. The organic phosphorus decreased in ten pots; increased in one pot, and was almost the same as the control in the other pot when cowpea cropped soils are considered. When tomato was used, there was a decrease in nine pots while there was an increase in the remaining three pots. With rice, there was again a decrease in organic phosphorus content in nine pots;

TABLE 1: ORGANIC PHOSPHORUS EXTRACTED FROM ORIGINAL SOIL AND FROM SOIL SAMPLES TAKEN AFTER THE 1ST GROWTH OF CROPS ON SOILS IN THE GREENHOUSE AND THE GAIN OR LOSS IN ORGANIC PHOSPHORUS OF CROPPED SOILS AS COMPARED WITH THE UNCROPPED (CONTROL) SOIL

Organic phosphorus extracted from soils ($\mu\text{g}/\text{gm}$ soil) after the 1st cropping period							The Gain (+) or loss(-) in organic phosphorus content of cropped soils as compared with the control				
POT No.	(A) MAIZE	(B) COWPEA	(C) TOMATO	(D) RICE	(S) CONTROL	(O) ORIGINAL	O-S	S-A	S-B	S-C	S-D
1	380.3	295.7	372.6	369.3	333.8	328.3	+ 5.5	+ 51.0	+ 33.6	+ 43.3	+ 40.0
2	386.7	341.5	387.7	329.8	333.8	328.3	+ 5.5	+ 57.4	+ 12.2	+ 58.4	+ 0.5
3	271.6	212.7	206.9	327.1	324.8	328.3	- 3.5	- 57.7	- 116.6	- 122.4	- 2.2
4	312.7	316.9	175.3	326.2	324.8	328.3	- 3.5	- 16.6	- 12.4	- 154.0	- 13.1
5	286.9	286.4	192.7	230.1				- 42.4	- 42.9	- 136.6	- 99.2
6	326.9	322.3	174.7	144.2				- 2.4	- 7.0	- 154.6	- 185.1
7	274.4	274.0	277.6	235.7				- 53.9	- 55.3	- 51.7	- 93.6
8	216.8	292.9	302.2	251.9				- 112.5	- 36.4	- 27.1	- 77.4
9	249.1	259.6	280.5	309.4				- 80.2	- 70.7	- 48.8	- 20.9
10	288.4	329.4	374.7	278.3				- 40.9	+ 0.1	+ 45.4	- 51.0
11	281.4	319.2	255.6	337.3				- 47.9	- 10.1	- 73.7	+ 6.0
12	265.2	263.8	263.3	295.4				- 64.1	- 65.5	- 66.0	- 33.9
MEAN	295.2	292.9	272.0	285.4	329.3	328.3	+ 1.0	- 34.1	- 36.4	- 57.3	- 43.9

two pots had an increase in organic phosphorus, and the other one had the same organic phosphorus content as the control. When the mean values are compared, it is observed, that there was a decrease in organic phosphorus content of all the cropped soils; the decrease being 34.1, 36.4, 57.3 and 43.9 ugP, in maize, cowpea, tomato and rice soils respectively.

The data presented in table 2 indicate that the organic phosphorus contents of all the maize pots did show a decrease when compared with the control. Eleven cowpea pots had lower organic phosphorus values than the control, while the remaining pot had a higher organic phosphorus than the control. There was a decrease in organic phosphorus content in all the tomato pots, and in contrast, the organic phosphorus in all the rice pots did show an increase. There was practically no difference between the organic phosphorus contents of the control in the 1st cropping period and that in the second cropping period. When the mean organic phosphorus values are compared with the control, it is observed that the organic phosphorus of maize, cowpea, tomato cropped soils decreased by 51.9, 61.6, and 138.2 ug P respectively. However, the organic phosphorus of rice cropped soil increased by as much as 125.7 ug P.

The data given in table 3 show that the organic phosphorus content of the nine pots for maize increased more than that of the control after the end of the 3rd cropping period. Three pots however had lower organic phosphorus values than the control. The organic phosphorus increased in all the cowpea pots. With tomato and rice, only two pots of each crop

TABLE 2

ORGANIC PHOSPHORUS EXTRACTED FROM UNCROPPED AND CROPPED SOILS SAMPLES TAKEN AFTER THE 2ND GROWTH OF CROPS ON SOILS USED PREVIOUSLY IN THE 1ST GROWTH PERIOD AND THE GAIN OR LOSS IN ORGANIC PHOSPHORUS OF CROPPED SOILS AS COMPARED WITH THE CONTROL (S₂) IN THE 2ND PERIOD; AND THE GAIN OR LOSS IN ORGANIC PHOSPHORUS IN THE CONTROL (S₂) AS COMPARED WITH CONTROL (S) IN 1ST PERIOD

Organic phosphorus extracted from soils ($\mu\text{g}/\text{gm}$ soil) after the 2nd cropping period							The Gain (+) or Loss (-) in organic phosphorus of cropped soils as compared with the control (S ₂) and in the control (S ₂) as compared with control (S) of the 1st cropping period					
POT	(A)	(B)	(C)	(D)	(S)	(S ₂)	S-S ₂	S ₂ -A	S ₂ -B	S ₂ -C	S ₂ -D	
NO	MAIZE	COWPEA	TOMATO	RICE	CONTROL	CONTROL						
1	329.5	300.7	194.4	393.8	333.8	341.3	+ 7.5	- 2.6	- 31.4	-137.7	+ 61.7	
2	323.9	324.9	224.0	480.9	333.8	341.3	+ 7.5	- 8.2	- 7.2	-108.1	+148.8	
3	262.5	289.7	183.7	484.1	324.8	322.8	- 2.0	- 69.6	- 42.4	-148.4	+152.0	
4	290.6	338.3	157.5	499.4	324.8	322.8	- 2.0	- 41.5	+ 6.2	-174.6	+167.3	
5	285.0	292.2	137.9	368.2				- 47.1	- 39.9	-194.2	+ 36.1	
6	288.2	238.6	103.3	390.2				- 43.9	-103.5	-238.8	+ 58.1	
7	286.0	286.9	269.8	472.5				- 46.1	- 45.2	- 63.3	+140.4	
8	215.8	271.6	269.7	376.8				-116.3	- 60.5	- 63.4	+ 44.7	
9	234.4	234.3	207.2	445.1				- 97.7	- 97.8	-124.9	+113.0	
10	293.7	233.2	176.1	407.9				- 38.4	- 98.9	-156.0	+ 75.8	
11	286.7	215.0	215.6	576.2				- 45.4	-117.1	-116.5	+244.1	
12	266.7	220.7	184.2	597.8				- 65.4	-111.4	-147.9	+265.7	
MEAN	280.2	270.5	193.9	457.8	329.3	332.1	+ 2.8	- 51.9	- 61.6	-138.2	+125.7	

TABLE 3 : ORGANIC PHOSPHORUS EXTRACTED FROM UNGROPPED AND CROPPED SOIL SAMPLES TAKEN AFTER THE 3RD GROWTH OF CROPS ON SOILS USED PREVIOUSLY IN THE 2ND PERIOD AND THE GAIN OR LOSS OF ORGANIC PHOSPHORUS OF CROPPED SOILS AS COMPARED WITH THE CONTROL (S₃) OF THIRD PERIOD AND THE LOSS IN THE CONTROL (S₃) AS COMPARED WITH CONTROL (S₂) IN THE 2ND PERIOD

The organic phosphorus extracted from soils (ug/gm soil) after the third cropping period							The Gain (+) or Loss (-) in organic phosphorus of cropped soils as compared with control (S ₃) and in the control (S ₃) as compared with the control (S ₂) of the 2nd cropping period				
POT No.	(A) MAIZE	(B) COWPEA	(C) TOMATO	(D) RICE	(S ₂) CONTROL	(S ₃) CONTROL	S ₂ -S ₃	S ₃ -A	S ₃ -B	S ₃ -C	S ₃ -D
1	333.3	324.4	432.2	305.9	341.3	304.7	-36.6	+ 31.8	+ 22.9	+130.7	+ 4.4
2	334.0	345.0	423.9	321.5	341.3	304.7	-36.6	+ 32.5	+ 43.5	+122.4	+ 20.0
3	311.5	326.2	383.2	345.5	322.8	298.3	-24.5	+ 10.0	+ 24.7	+ 81.7	+ 44.0
4	306.7	386.1	349.4	322.7	322.8	298.3	-24.5	+ 5.2	+ 84.6	+ 47.9	+ 21.2
5	332.7	436.4	385.7	298.0				+ 31.2	+134.9	+ 84.2	- 3.5
6	317.5	437.7	369.8	363.6				+ 16.0	+136.2	+ 68.3	+ 62.1
7	282.8	419.2	320.7	295.5				- 18.7	+117.7	+ 19.2	- 6.0
8	365.2	377.4	348.3	377.0				+ 63.7	+ 75.9	+ 46.8	+ 75.5
9	380.7	378.0	248.2	354.4				+ 70.2	+ 76.5	- 53.3	+ 52.9
10	342.3	330.5	325.2	332.5				+ 40.8	+ 29.0	+ 23.7	+ 31.0
11	281.9	404.2	345.5	342.8				- 19.6	+102.7	+ 44.0	+ 41.3
12	292.6	393.0	246.6	372.8				- 8.9	+ 91.5	- 54.9	+ 71.3
MEAN	323.4	379.8	348.3	336.0	332.1	301.5	-30.6	+ 21.9	+ 78.3	+ 46.8	+ 34.5

had lower organic phosphorus values than the control; the rest had higher values than the control. When the mean organic phosphorus values are compared, it is found that the organic phosphorus increased in all the cropped soils more than in the control. The largest increase in organic phosphorus was found in cowpea cropped soils an increase of 78.3ugP and the smallest increase in maize cropped soil, an increase of 21.9 ugP. The organic phosphorus increased by 46.8 ugP in tomato cropped soil, and in the rice cropped soil; the increase was 34.5 ugP. When the organic phosphorus values of the controls in both 2nd and 3rd cropping periods are compared, it is noted that the organic phosphorus decreased by 30.6 ugP in the 3rd cropping period.

A comparison was made of the data on organic phosphorus of the cropped soils during the three cropping periods. The analysis of variance is shown in table 4.

Table 4. Analysis of variance of data on organic phosphorus found in four cropped soils during the three cropping periods.

Source of Variation	Degrees of freedom	Sum of squares	Mean square	F
Crops	3	110224.71	36741.57	13.91 **
Croppings	2	308642.24	154321.12	58.46 **
Cropping x Crops	6	276957.21	46159.54	17.48 **
Error	132	348472.98	2639.95	
Total	143	1044297.14		

* * Significant at 1 per cent level.

The least-significant-difference (L.S.D.) test was next applied to the data. In this test, the difference between two means is said to be significant if it exceeds the value derived for the L.S.D. at the desired level of significance.

The LSD values derived at the 5 per cent and 1 per cent levels for the crops are 20.77 and 27.45 respectively. Below are the means of organic phosphorus for the four cropped soils ranked from the highest to the lowest.

<u>Rice</u>	<u>Cowpea</u>	<u>Maize</u>	<u>Tomato</u>
359.4	314.4	299.6	271.4

Thus it appears from the LSD test that the growth of rice increased the organic phosphorus content of soil more than cowpea both at the 5 percent and 1 percent levels. The difference between the means of organic phosphorus values for cowpea and maize cropped soils is not significant at 5 percent level. The difference between means of organic phosphorus of maize and tomato soils is significant at both 5 percent and 1 percent levels.

The LSD values derived at the 5 per cent and 1 per cent levels for cropping periods are also 23.92 and 31.61 respectively. The means of organic phosphorus content for the cropping periods ranking from the highest to the lowest are as follows:

<u>3rd Cropping Period</u>	<u>2nd Period</u>	<u>1st Period</u>
346.9	300.6	286.4

The test indicates that the difference between the means of organic phosphorus of third and second cropping periods, is significant at 1 per cent level. The difference between means of second and first periods is not significant at 1 per cent level, but significant at 5 per cent level.

The means of organic phosphorus of crops within each cropping period are ranked from highest to lowest as follows:

	<u>Maize</u>	<u>Cowpea</u>	<u>Rice</u>	<u>Tomato</u>
1st Period	295.2	292.9	285.4	272.0
	<u>Rice</u>	<u>Maize</u>	<u>Cowpea</u>	<u>Tomato</u>
2nd Period	457.8	280.2	270.5	193.9
	<u>Cowpea</u>	<u>Tomato</u>	<u>Rice</u>	<u>Maize</u>
3rd Period	379.8	348.3	336.0	323.4

After the 1st cropping period, there are no significant differences between the means of organic phosphorus of cropped soils. After the 2nd cropping period, there is highly significant difference between the means of rice and maize cropped soils at 1% level. However the means of the maize and cowpea cropped soils do not show any significant difference. There is highly significant difference between means of cowpea and tomato cropped soils. After the third period, the

difference between cowpea and tomato cropped soils shows significance at 1% level. However there are no significant differences between means of tomato and rice, and those of rice and maize cropped soils.

In table 5, it is observed that after the first cropping period, a maize pots had percentage organic carbon values lower than the control; the remaining pots had higher percentage organic carbon values than the control. Cowpea, rice and tomato pots had lower in % organic carbon than the control. The differences between the means of the cropped soils and that of the control were very small indeed, differences of 0.1 to 0.4%. It can be observed from tables 5, 6 and 7 that the mean values of the maize soils after the three cropping periods showed a small difference of 0.1%, while in cowpea, rice and tomato soils, there was practically no change in the mean values throughout the cropping periods. The control of the 1st period had a higher value than the original soil. The difference between values of the controls was 0.2%

In general, there was no significant difference between the organic carbon percentage values of the uncropped (controls) and the cropped soils during the cropping periods. No statistical analyses were made on the values because it is obvious from the results that differences between the crops and the cropping periods are not significant.

Table 5. Organic Carbon contents of original soil, Cropped and uncropped soils after the 1st period and the gain or loss of organic carbon of the cropped and uncropped soils.

Organic carbon percentages of soils after the 1st cropping period							Gain (+) or loss (-) organic carbon of the cropped and the uncropped soils.				
Pot No	A Maize	B Cowpea	C Tomato	D Rice	S Control	O Original	O-S	S-A	S-B	S-C	S-D
1	2.8	2.8	2.8	2.7	3.1	2.9	+ 0.2	- 0.3	- 0.3	- 0.3	- 0.4
2	2.9	2.7	2.9	2.8	3.1	2.9	+ 0.2	- 0.2	- 0.4	- 0.2	- 0.3
3	3.2	2.7	2.7	2.8	3.0	2.9	+ 0.1	+ 0.1	- 0.4	- 0.4	- 0.3
4	3.2	2.9	2.6	2.7	3.0	2.9	+ 0.1	+ 0.1	- 0.2	- 0.5	- 0.4
5	3.2	2.8	2.6	2.6				+ 0.1	- 0.3	- 0.6	- 0.5
6	3.5	2.9	2.5	2.7				+ 0.3	- 0.2	- 0.3	- 0.4
7	2.9	2.8	2.8	2.7				- 0.2	- 0.3	- 0.3	- 0.4
8	2.6	2.8	2.8	2.6				- 0.5	- 0.3	- 0.3	- 0.5
9	2.5	2.7	2.8	2.6				- 0.6	- 0.4	- 0.3	- 0.5
10	2.6	2.7	2.7	2.8				- 0.5	- 0.4	- 0.4	- 0.3
11	2.7	2.8	3.0	2.8				- 0.4	- 0.3	- 0.1	- 0.3
12	2.5	2.8	2.7	2.8				- 0.6	- 0.3	- 0.4	- 0.3
Mean	2.9	2.8	2.7	2.7	3.1	2.9	+ 0.2	- 0.2	- 0.3	- 0.4	- 0.4

Table 6 : The percentage organic carbon of cropped and uncropped soils, and the gain or loss in organic carbon of the soils after the 2nd period.

Organic carbon percentages of cropped, and uncropped soils after the 2nd cropping period							Gain (+) or loss (-) in organic carbon of the cropped and uncropped soils.				
Pot No	A Maize	B Cowpea	C Tomato	D Rice	S Control	S ₂ Control	S-S ₂	S ₂ -A	S ₂ -B	S ₂ -C	S ₂ -D
1	2.6	2.8	2.8	2.8	3.0	2.8	- 0.2	- 0.3	- 0.1	- 0.1	- 0.1
2	2.6	2.9	2.9	2.8	3.0	2.9	- 0.1	- 0.3	--	--	- 0.1
3	2.6	2.7	2.7	2.8	3.1	2.9	- 0.2	- 0.3	- 0.2	- 0.2	- 0.1
4	2.7	2.8	2.8	2.7	3.1	2.8	- 0.3	- 0.2	- 0.1	- 0.1	- 0.2
5	2.9	3.0	2.7	2.8				--	+ 0.1	- 0.2	- 0.1
6	2.8	2.9	2.8	2.8				- 0.1	- 0.1	- 0.1	- 0.1
7	2.6	2.6	2.7	2.7				- 0.3	- 0.2	- 0.2	- 0.2
8	2.6	2.8	2.8	2.6				- 0.3	- 0.1	- 0.1	- 0.3
9	2.7	2.7	2.7	2.7				- 0.2	- 0.2	- 0.2	- 0.2
10	2.7	2.8	2.8	2.7				- 0.2	- 0.1	- 0.1	- 0.2
11	2.7	2.7	2.8	2.7				- 0.2	- 0.2	- 0.1	- 0.2
12	2.7	2.9	2.8	2.8				- 0.2	--	- 0.1	- 0.1
Mean	2.7	2.8	2.8	2.7	3.1	2.9	- 0.2	- 0.2	- 0.1	- 0.1	- 0.2

Table 7 : The percentage organic carbon of cropped and uncropped soils, and the gain or loss in organic carbon of the soils after the 3rd period.

Organic carbon percentages of cropped, and uncropped soils after the 3rd cropping period							Gain (+) or Loss (-) in organic carbon of the cropped and uncropped soils.				
Pot No	A Maize	B Cowpea	C Tomato	D Rice	S ₃ Control	S ₃ Control	S ₂ -S ₃	S ₃ -A	S ₃ -B	S ₃ -C	S ₃ -D
1	2.6	2.9	2.7	2.9	2.8	2.8	—	- 0.1	+ 0.2	—	+ 0.2
2	2.8	2.7	2.6	2.7	2.9	2.7	- 0.2	+ 0.1	—	- 0.1	—
3	2.8	2.7	2.6	2.8	2.9	2.8	- 0.1	+ 0.1	—	- 0.1	+ 0.1
4	2.8	2.7	2.7	2.7	2.8	2.7	- 0.1	+ 0.1	—	—	—
5	2.7	2.8	2.5	2.6				—	+ 0.1	- 0.2	- 0.1
6	2.7	2.7	2.7	2.8				—	—	—	+ 0.1
7	2.9	2.8	2.7	2.6				+ 0.2	+ 0.1	—	- 0.1
8	2.8	2.8	2.7	2.6				+ 0.1	+ 0.1	—	- 0.1
9	2.9	2.8	2.7	2.6				+ 0.2	+ 0.1	—	- 0.1
10	2.6	2.6	2.7	2.8				- 0.1	- 0.1	—	+ 0.1
11	2.8	2.8	2.6	2.7				+ 0.1	+ 0.1	- 0.1	—
12	2.8	3.0	2.7	2.7				+ 0.1	+ 0.3	—	—
Mean	2.8	2.8	2.7	2.7	2.9	2.7	- 0.1	+ 0.1	+ 0.1	—	—

The data of the experiment on the effects of different volumes of both unheated and heated extracts on disodium phenylphosphate are given in table 8. Each value is an average of two determinations. Unheated extract from tomato seedlings produced the highest amount of inorganic phosphorus with increasing volume. The lowest amount of inorganic phosphorus produced was by unheated rice extract. It can be observed from the data of unheated extracts that the activity of the extracts increased almost linearly with increasing volume, and hence the concentration of the 'enzyme' extracts.

Table 8. Inorganic phosphorus produced from the action of different volumes of heated and unheated 'enzyme' extracts from maize, cowpea, tomato and rice roots on disodium phenylphosphate.

Volume of Extracted added(mls)	ugP/10ml. aliquot used							
	Maize		Cowpea		Tomato		Rice	
	unheated	heated	unheated	heated	unheated	heated	unheated	heated
5	5.45	2.1	2.4	2.7	7.0	2.8	2.7	2.6
10	9.57	2.8	3.7	2.6	11.9	2.7	2.3	2.5
20	15.2	1.3	5.8	2.5	17.5	3.6	3.7	2.6
25	18.2	3.2	7.4	2.7	19.4	4.1	5.5	2.2

The data on the inorganic phosphorus produced by heated extracts indicated that heating had a significant effect on the activity of the extracts. The activities of the heated extracts did not increase linearly, but remained almost constant with increasing volume. Tomato and maize extracts were greatly affected by heating, whereas cowpea and rice extracts were least affected by heating.

Table 9 contains the results of the effect of the 'enzyme' extracts on soil organic phosphorus. There were no significant differences between the control and soil plus 'enzyme' extracts. There was little or no difference in soil organic phosphorus between treatments with toluene and treatments without toluene. However, soil plus tomato extract with and without toluene treatments had the lowest organic phosphorus content. The organic phosphorus of treatments with toluene and without toluene were 306.4 and 310.4 ugP/gm soil respectively.

Table 9 : Organic phosphorus content of soil after incubation with different 'enzyme' extracts

Treatment	Organic phosphorus found in culture gm of soil. ug.	
	No toluene	Toluene
Dry soil	324.3	325.2
Soil + water	319.5	322.6
Soil + maize extract + water	326.8	325.6
Soil + cowpea extract + water	325.5	326.9
Soil + tomato extract + water	310.4	306.4
Soil + rice extract + water	324.7	323.2

DISCUSSION

The results of the greenhouse experiment presented in tables 1, 2 and 3 showed that with the exception of rice cropped soil which showed an increase in organic phosphorus content after the second cropping period, all the cropped soils did show a decrease after the first and second cropping periods. After the third cropping period, the organic phosphorus increased in all the cropped soils more than the control. The almost constant organic phosphorus content of the controls throughout the investigation indicated that any change in organic phosphorus of cropped soils was the result of the action of growing plants. The results, so far outlined, are conflicting, but perhaps they can be explained.

The results of the first and second cropping periods, with the exception of rice, agreed with findings of many investigators. Vincent (1937), Hayashi and Takijima (1955), Sekhon and Black (1969) and others observed that the total organic phosphorus content of soil decreased during the growth of a crop. Williams (1950) noted that the organic phosphorus soluble in dilute NaOH did decrease during the growth of a crop on a soil. The results of the experiments conducted by Thompson and Black (1970) also revealed a decrease in soil organic phosphorus during cropping. Thompson (1967), investigating the short-term effects of plants on soil organic phosphorus observed a decrease

in the organic phosphorus.

The decrease in organic phosphorus observed during the two growing periods might have been caused by increased mineralization of the soil organic phosphorus by some substances secreted by the plants or by direct absorption of the organic phosphorus by the plants. To verify that the plants in fact produced substances capable of hydrolyzing the organic phosphorus in the soil, an investigation was then carried out. The results obtained from the investigation and presented in table 8 indicated that the plant roots showed phosphatase activity and this activity varied from crop to crop. The results were in agreement with those of Rogers et al (1940), Rovira (1959), Estermann and McLaren (1961) and many others. Rovira (1959) and Rogers et al (1940) observed a vigorous phosphatase activity of corn and tomato roots. Hayashi and Takijima (1953) noted that phosphatase activity, particularly that of glycerophatase, was higher than the activity of other constituents in roots of phosphorus -deficient cereals. Jensen (1956), and McGregor and Street (1953), located phosphatase in the roots of some crops.

A decrease in soil organic phosphorus induced by plants after the two cropping periods has now been reported in the investigation. Furthermore, production of phosphatase enzyme by the test crops has

also been established. Therefore, if an increase in rate of mineralization of soil organic phosphorus due to enhanced phosphatase activity in the presence of plants was responsible for the decrease, similar additions of 'enzyme' extracts should produce a decrease in soil organic phosphorus in the absence of plants. In this investigation additions of the root extracts were made to untreated soil. The results given in table 9 indicated that additions of root extracts did not cause any change in organic phosphorus, a conflict with the phosphatase activity Hypothesis. Failure to find a phosphatase-induced decrease in soil organic phosphorus can be explained by some deficiency in the technique, resistance of soil organic phosphorus to mineralization by the phosphatase extracts, or by low concentrations of phosphatase in the extracts.

The rapid increase in organic phosphorus of the soil in the presence of rice plants after the second cropping period may be attributed to the growth of fungi and algae on the surface of soils in which rice seedlings were planted. It was interesting to observe that only pots in which rice seedlings were planted during the second cropping period had algae and fungi growing in them. The presence of these microbes increased the microbial population which in turn might have increased the organic phosphorus content. This observation was similar to that found by Thompson (1967).

After the third cropping period, all the cropped soils had higher organic phosphorus than the control. This observation agreed with one of the experiments conducted by Thompson (1967) who found that the loss of organic phosphorus in the control was greater than the loss from soil in the presence of tomato and corn plants. The increase in organic phosphorus content of the cropped soils may be explained on the basis that the presence of the plants caused addition of organic phosphorus directly to the soil or secretion of certain organic substances which caused an increase in numbers of microbes in the soil. It is believed that the roots of crops have a considerable microbial population on or close to their surface and the food supply of this rhizosphere population is presumably either the root excretions, sloughed-off root cells, root hairs or organic substances the surface population extracts from the living roots. This population assimilate these substances into their cells thereby the organic phosphorus of their cells increases, thus affecting the overall content of organic phosphorus of the soil. Another cause of the increase in organic phosphorus of the soil might also be the direct contribution by sloughed-off root tips in the soil where the environmental conditions prevailing might be conducive as to effect a rapid decomposition of the roots. However, the contribution which might have been made by the broken roots may be very small indeed, because the contribution has been

corrected for by using sand controls in the experiment.

The loss after the first two cropping periods and the build up after the third cropping period of the soil organic phosphorus and the production of phosphatase by crops suggest that both immobilization and mineralization by enhanced phosphatase activity of organic phosphorus are occurring concurrently in the soil. However, failure to find a decrease in soil organic phosphorus by phosphatase extracts indicates that the possibility of direct absorption of organic phosphorus by plants cannot completely be ruled out and it needs further examination.

Because of the approximately constant percentage of carbon in humus, it can be used as an index of the amount of organic matter present in the soil. The observation of almost constant organic matter content of cropped and the uncropped soils during the investigation confirmed the findings of Coutler (1950), Shutt (1925), Blair and Prince (1936) who observed that, over a short cropping period, the organic matter of the soil remained constant. This almost constant organic matter of the cropped soils, may be explained on the basis that the humus present in the soils was more or less "inert" and could not undergo any decomposition under the prevailing conditions.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The purpose of the investigation was to determine whether successive cropping caused a change in the organic phosphorus and organic matter contents of soil, and to determine the cause of the change in the organic phosphorus. The soil used in the investigation was selected particularly for its relatively high organic matter and organic phosphorus content, so that any change in these factors during the period of the experiment could be detected. Maize, cowpea, tomato and rice were used as test crops.

In the greenhouse experiment, soils were cropped for 30 days. In the case of tomato, the seeds were nursed in sand in trays for two weeks during which the seedlings were supplied with Hoagland solution of 1/5 strength. The seedlings were then transplanted into soil and grown for 35 days. To correct for the organic phosphorus which might be contributed by broken roots, similar experiment using sand was set up. The soil and the sand were cropped thrice using the same soil or sand for the same test crop. Controls, that is soil or sand with no plants were also set up. At the end of each growing period, the soil and the sand were analysed for organic phosphorus

and organic carbon using Mehta et al (1954) and Walkley and Black (1934) methods respectively.

The results of the soil after the correction of the sand values indicated that the organic phosphorus of the cropped soils decreased more than the uncropped soil after the two cropping periods. The loss of organic phosphorus of cropped soil of tomato was greater than those of maize and cowpea cropped soils. On the contrary, there was a rapid increase in organic phosphorus of rice cropped soil during the second cropping period. This rapid increase has been attributed to the growth of fungi and algae on the rice cropped soil. There was no significant change in the organic phosphorus content of uncropped soil during the experiment. After the third cropping period, there was a build up of organic phosphorus of all the cropped soils, with cowpea cropped soil showing the largest increase. The probable explanation to this build up of organic phosphorus of the cropped soil is that, the plants secreted either organic phosphorus substance directly or certain organic substances into the soil which caused an increase in the microbial population and hence increase in organic phosphorus content of the soil.

To verify that the decrease of the organic phosphorus content of soil in the presence of plants observed after the first two cropping periods was the result of enhanced phosphatase activity by

the roots of the plants, supplementary experiments were then carried out. Seedlings were grown in distilled water in growth chamber for one week and the extracts were added to a known amount of organic phosphorus in the form of disodium phenylphosphate and incubated for 20 hours with shaking at room temperature. After the incubation, the inorganic phosphorus of the aliquot was determined by Dickman and Bray (1940) method. The results showed that the roots of all the plants produced an 'enzyme' phosphatase which hydrolysed part of the organic phosphorus substrate. Tomato plants exhibited the greatest phosphatase activity, whilst rice seedlings showed the least activity. The activity also increased linearly with increasing volume, and hence the concentration.

To test further that the substance which caused the hydrolysis of the organic phosphorus was indeed an enzyme, the extracts were heated for 3 hours at 90°C. Different volumes of the heated extracts were incubated with the organic phosphorus compound. Inorganic phosphorus was also determined after the incubation period. The evidence obtained in the experiment indicated that heating reduced the activities of the plants.

The observation of phosphatase activity by the test crops indicated that the most likely cause of the decrease in organic phosphorus content of the soil due to the presence of plants seemed

to be an increase in rate of mineralization of soil organic phosphorus associated with enhanced phosphatase activity in the soil around the roots. The experiment on the addition of the extracts to the soil in the absence of plants showed that the extracts did not change the organic phosphorus content of the soil. The results did not support the hypothesis that enhanced phosphatase activity in the soil caused by the roots was responsible for the decrease in soil organic phosphorus in the presence of plants and therefore an examination of the direct absorption hypothesis seems proper.

The organic matter content of both the cropped and the uncropped soils remained unchanged during the cropping periods, an observation which confirms the general belief that cropped soils in the short-run do not lose their organic matter and that the humus in the soil was rather "inert" and therefore could undergo any further decomposition under the prevailing conditions.

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