The Movement of Phytic Acid in Soil Cores[1]

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ABSTRACT

The movement of phytic acid-phosphorus in a vertical soil core was studied under controlled conditions using a miscible displacement technique. Experiments were conducted on a sandy loam soil with gamma irradiation at 20C and without irradiation at 5C and 20C. Phytic acid appeared to be quickly bound by the soil, accumulating near the top of the soil core. Phytic acid apparently displaced native inorganic phosphorus. The movement of phytic acid increased and its rate of mineralization decreased at 5C as compared to 20C. Gamma irradiation reduced, but did not completely eliminate, mineralization of phytic acid at 20C. Changes in free iron concentration within the core were noted and related to the movement of phytic acid-phosphorus.

Additional Key Words for Indexing: phosphorus movement, organic phosphorus, mineralization, iron movement.

ORGANIC PHOSPHORUS compounds in the soil have been shown to exhibit distributions within soil profiles similar to that of the organic matter in general (2, 9, 15, 17). The occurrence of organic compounds in the deeper soil horizons may be a result of movement of these compounds down the profile, or a biological synthesis of organic compounds *in situ.* Attempts to leach inorganic phosphorus through a soil core resulted in an immobilization of the phosphorus that was related to microbial activity (16). A similar reaction in a soil profile could result in the synthesis or organic phosphorus compounds in the lower horizons from mobile inorganic phosphorus (3).

The movement of organic phosphorus compounds in soil has received little attention. The studies of Hannapel et al. (11, 12) have indicated that organic phosphorus would move through a soil under certain conditions. This movement appeared to be related to the activity of microorganisms. These studies were carried out under unfavorable conditions as the soil columns were saturated. Movement techniques have been developed that permit control of the water content of the soil (7, 8). These techniques offer the advantage of a more natural soil condition.

This study was initiated to investigate the movement of phytic acid, the major identified organic phosphorus compound in soil, employing techniques designed to control microbial activity, temperature, water content and flow rate.

MATERIALS AND METHODS

Phytic acid was prepared from a commercial sample of phytin (calcium-magnesium inositol hexaphosphate) using a procedure based on the methods of Anderson (1) and Pedersen (18). The phytic acid solution used in all the following experiments contained 505 ppm organic P and 20 ppm inorganic P. Qualitative paper chromatography yielded only one major organic phosphorus compound.

Phytic acid (inositol hexaphosphate) solution was flushed through a 15.24 cm long by 3.81 cm diam (6 by 1.5 in.) vertical core of Fox sandy loam (pH 7.35, clay 7.31%, silt 17.49%, sand 75.2%, bulk density 1.35 g/cc), using a miscible displacement technique (7, 8). The height of the constant head burette containing the tracer (phytic acid) above the soil core (Fig. 1) determined the flow rate through the core. The flow rate was normally maintained at 3 to 5 ml/hr (1 ml of effluent is equivalent. to 0.088 cm of water leached). A positive air pressure of 50 cm of water was applied externally around the perforated plexiglas cylinder containing the soil to control the water content of the soil core below saturation. However, the actual water content was not determined.

Experiments were conducted at 20C with and without gamma irradiation of the soil, and at 5C without gamma irradiation. The treated soil was irradiated sufficiently to sterilize it (3 megarads) before the core was packed. The irradiated soil was packed into a sterilized core using aseptic techniques to minimize microbial contamination.

The effluent was collected automatically in 5-ml fractions and analyzed for total and inorganic phosphorus. At the termination of each experiment, the soil core was immediately frozen, cut into 6 equal segments and lyophilized. Analyses were conducted on each segment to determine the total and inorganic phosphorus content, the free iron content, and the percent sand, silt and clay. Phosphorus analyses were conducted using the ascorbic acid reductant method of Colwell (6) for the Technicon AutoAnalyzer after extraction of the core sections with Dowex A-1 resin (19). Free iron was extracted using the method of Coffin (5) and colorimetrically determined using the method of Jackson (14). Particle size distribution was determined by the pipette method.

The effluent solutions were examined qualitatively by paper chromatography for the presence of phytic acid. High concentrations of phytic acid were detected, but no attempt was made to estimate what portion of the organic phosphorus was actually phytic acid.

RESULTS AND DISCUSSION
Experiment 1

The plexiglas cylinder (Fig. 1) was packed with 243 g of soil and vacuum-saturated with distilled water prior to introducing the phytic acid solution. The temperature in this experiment was maintained at 5C.

Inorganic phosphorus first appeared after 300 ml of effluent had been collected (Fig. 2) and reached a maximum of 53 ppm after collecting 1,400 ml. For the remainder of the experiment the inorganic phosphorus concentration decreased slowly. No explanation can be offered for the sharp peak at about 4,500 ml, unless it is a response to the start of the water wash. Organic phosphorus first appeared after collecting 500 ml of effluent (Fig. 3) and rapidly increased. After the 700-ml point, fine white material appeared in the effluent. Analyses indicated that it contained about 10 to 16% organic phosphorus as well as an undetermined amount of iron.
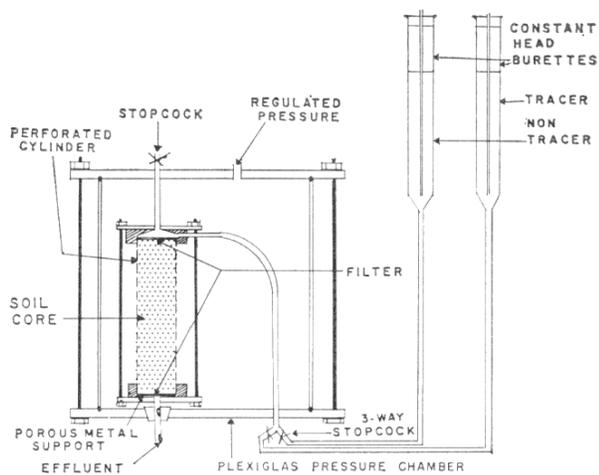
Fig. 1—Schematic diagram of miscible displacement apparatus.
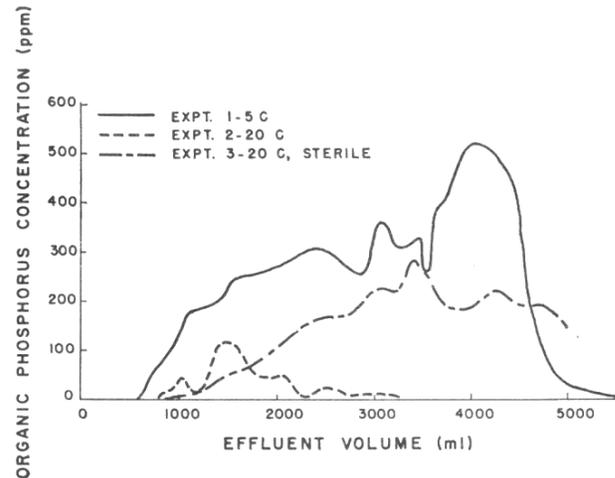
Fig. 3—Elution pattern of organic P from soil cores.
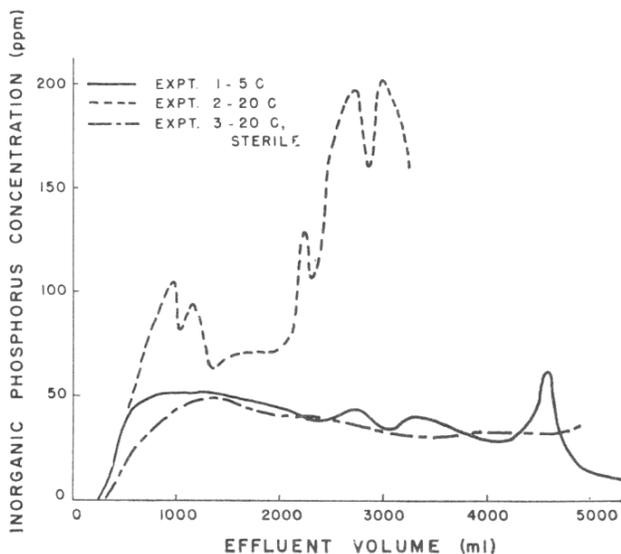
Fig. 2—Elution pattern of inorganic P from soil cores.

Because of the difficulty of evenly distributing the white material throughout each fraction before sampling, the organic phosphorus concentrations appeared to fluctuate considerably. It is believed that this white material consisted largely of ferric phytate.

After collecting 4,440 ml of effluent, the flow of phytic acid was cut off and about 1,050 ml of water was flushed through the core in order to obtain a release curve for phosphorus. The analysis of the core for retained phosphorus showed that the concentration of organic phosphorus increased from 630 µg/g soil at the top of the core to 5,450 µg/g soil in the fifth segment (Fig. 4a). Since the phytic acid phosphorus entered the top of the core, one would expect its concentration to be greatest there, decreasing toward the exit end. These results indicate that the water wash transported phosphorus toward the base of the core. Figure 4b shows that the concentration of inorganic phosphorus which will include both native and added inorganic phosphorus increased slightly from the top to the base of the core. The increase in inorganic phosphorus with depth in the core was attributed to its displacement at the upper end of the core by phytate salts which are less soluble in the soil than inorganic phosphorus (13). The earlier appearance of inorganic phosphorus in the effluent (Fig. 2 and 3) also indicates that the phytic acid dissolved or displaced inorganic phosphorus in the soil. Recovery values for organic and inorganic phosphorus in the core and effluent indicated that there had been a minimal amount of mineralization of organic phosphorus in the soil core at 5C.

Figure 4c shows the distribution of free iron in the soil core at the termination of the experiment. The dotted line represents the free iron content of the soil at the beginning of the experiment. Free iron appears to have moved downward in the core. However, the increase in free iron in the lower segments over the original soil was greater than the amount removed from the first segment. In preliminary experiments similar results were obtained. It is believed that the phytic acid enhanced the extractive power of the sodium dithionite for free iron, resulting in more iron being extracted from the soil in the presence of phytic acid. In addition to this apparent increase in free iron content in the soil core at the conclusion of the experiment, some iron was removed from the soil and appeared in the fine white material in the effluent.

Experiment 2

The core was packed with 246 g of soil and set up as shown in Fig. 1. The temperature in this experiment was regulated at 20C. Distilled water was flushed through the core for several hours prior to introducing the phytic acid solution.

As in Experiment 1, inorganic phosphorus first appeared in the effluent after collecting about 300 ml (Fig. 2). However, the inorganic phosphorus concentration increased to 100 ppm in this experiment. Organic phosphorus (Fig. 3) accompanied by the previously described white material appeared after collecting about 750 ml. Simultaneously the permeability decreased and the burette had to be raised to maintain the flow rate. At the 800-ml point, a considerable amount of white material appeared in the effluent, the flow rate suddenly increased, and the organic phosphorus concentration in the effluent suddenly increased.

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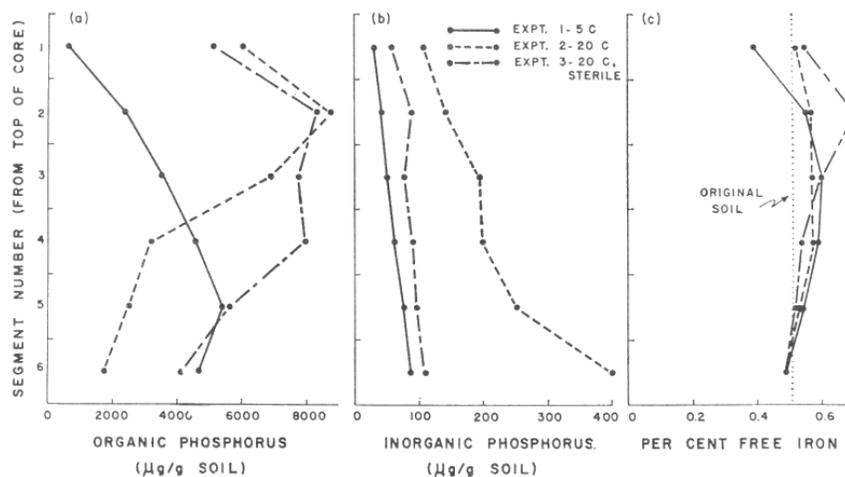
Fig. 4—Distribution patterns of organic P, inorganic P and free iron within soil cores after leaching.

The same cycle repeated itself in the 1,050- to 1,500-ml region. This time the rate remained at about 10 to 12 ml/hr for 11 hours, resulting in a considerable breakthrough of organic phosphorus. It may be noted that the inorganic phosphorus (Fig. 2) concentration in this region decreased. After readjusting the flow rate to 4 to 5 ml/hr, the organic phosphorus concentration decreased, and the inorganic phosphorus concentration increased until at the 2,000-ml point only inorganic phosphorus appeared in the effluent. From this point on, the phytic acid was being mineralized as fast as it entered the soil core.

It is believed that at the high flow rate, the microorganisms were unable to mineralize all the inflowing phytic acid, resulting in the observed increase in organic phosphorus concentration, and the decrease in inorganic phosphorus concentration. That is, the fraction of the phytic acid mineralized to inorganic phosphorus was reduced during the high flow rate period. However, when the flow rate was reduced, the microorganisms mineralized all the inflowing phytic acid resulting in only inorganic phosphorus appearing in the effluent throughout the terminal portion of the experiment. During the experiment an *Aspergillus* was isolated from the upper endpiece of the soil core, and from the inflow tubes. It is possible that this fungus was responsible for at least part of the mineralization of the phytic acid. Other workers have made similar findings (4,10).

The recovery value for organic phosphorus was only 77% in this experiment, indicating that more than 20% of the added organic phosphorus was mineralized. This figure for mineralization was probably low because mineralized phytic acid phosphorus that was incorporated into microbial cell tissue would be analyzed as organic phosphorus. This may partially account for the increase in the amount of organic phosphorus held in the soil in this experiment over that in Experiment 1.

In this experiment, no water wash followed the cut off of the phytic acid solution. Figure 4a shows that the organic phosphorus concentration was greatest near the top of the core, and decreased toward the bottom, unlike the results in the previous experiment where a water-wash was conducted. The lesser amount of organic phosphorus held in the first segment than in the second segment is believed to be a result of a partial destruction of the absorption capacity of the soil by the acidic phytic acid (pH 2.2). The inorganic phosphorus distribution (Fig. 4b) showed a marked increase toward the base of the soil core. Most of this increase is attributed to mineralized phytic acid which was displaced by the inflowing phytic acid solution.

As in the previous experiment, the free iron content of the soil seemed to increase after coming in contact with the phytic acid solution. Little explanation can be offered regarding the free iron movement except that some iron was incorporated into the white material found in the effluent.

Experiment 3

In order to minimize the effects of microorganisms upon the behaviour of phytic acid in the soil, the soil was irradiated with gamma rays (3 megarads) before initiating the experiment. The same apparatus was used as in the previous experiments, (Fig. 1) and the soil core was washed with distilled water for 2 days before introducing the phytic acid solution. This long prewash period was found to be necessary to remove organic matter solubilized by the gamma rays. Without this long prewash, the phytic acid quickly reacted with the solubilized material and blocked the flow channels, preventing further flow. The temperature was again maintained at 20C.

Inorganic phosphorus first appeared after collecting 330 ml of effluent (Fig. 2), and the first traces of organic phosphorus appeared at 700 ml (Fig. 3). The inorganic phosphorus declined from its peak concentration of 50 ppm at 1,250 ml while the organic phosphorus concentration slowly increased to a maximum of 283 ppm at about 3,400 ml. As in the previous experiments, the fluctuations in the organic phosphorus concentration in the effluent were caused by small variable amounts of the white material in the effluent. Approximately 5 liters of phytic acid were put into the core in this experiment. No water wash followed the cut off of the phytic acid solution, since in the first experiment both forms of phosphorus disappeared from the effluent almost as soon as the water wash started.

As in Experiment 2, the organic phosphorus concentration in the soil core was the greatest near the input end (Fig. 4a). Because of the large amount added, the phytic acid penetrated to the lower segments of the core. Again, the decreased amount of organic phosphorus held in the first segment was attributed to destruction of the absorptive capacity of the soil by the phytic acid solution. The inorganic phosphorus distribution (Fig. 4b) increased from 66 µg/g in the top segment to 109 µg/g soil in the bottom segment.

The recovery value for organic phosphorus in this experiment was 92% indicating that some mineralization had still occurred in spite of the lethal dosage of gamma irradiation. Free phosphatases present in the soil, and intracellular phosphatases released from microbial cells during irradiation may have been responsible for this limited amount of mineralization.

The soil core retained 1.727 g organic phosphorus in this experiment, as opposed to only 0.866 g organic phosphorus in Experiment 1 which was conducted at 5C. The amounts of added organic phosphorus were 2.032 g and 2.545 g respectively. Since microbial interferences were at a minimum in both experiments, it would appear that the soil can retain considerably more organic phosphorus at 20C, than at 5C. The water wash used in the first experiment would not likely have any significance with regard to these differences since very little organic or inorganic phosphorus appeared in the effluent after the water wash was started.

The free iron distribution (Fig. 4c) showed a sharp peak in segment 2. This large increase in extractable iron coincided with the region of high organic phosphorus concentration in the core (Fig. 4a). This again supports the hypothesis of a phytic acid effect upon the extraction of free iron. In another experiment, (not presented here), only a small amount of phytic acid was passed through a soil core. In this case, there was little or no change in extractable iron over the original soil.

SUMMARY AND CONCLUSIONS

Attempts were made to move phytic acid through cores of Fox sandy loam under different environmental conditions. Data collected in this study indicated that phytic acid was rapidly bound by the soil, resulting in limited movement. This rapid reaction can be attributed to the insoluble complex formed by the chelation of metallic cations in the soil by phytic acid (13). In the present study, iron seems to have reacted with phytic acid, producing a whitish material that appeared in the effluent.

Evidence obtained in this study supports earlier work (13) that inorganic phosphorus is more mobile in the soil than organic phosphorus. Phosphorus distributions in the soil cores showed an increase in inorganic phosphorus toward the base of the core, whereas organic phosphorus tended to accumulate in the upper portion of the core (providing there was no water wash). Since phytate forms a more insoluble precipitate with metallic cations than orthophosphate (13), phytate ions probably displaced inorganic phosphorus from compounds with metallic cations, resulting in the downward movement and subsequent appearance of inorganic phosᵇhorus in the effluent.

Temperature had a marked influence upon the ability of the soil to hold organic phosphorus. An increase in temperature from 5C to 20C resulted in a 2-fold increase in the amount of organic phosphorus held by the soil. The temperature increase also resulted in increased microbial activity, especially fungal activity as evidenced by the great proliferation of fungal mycelia in the soil core and the end pieces of the apparatus when run at 20C without gamma irradiation. A species of *Aspergillus* capable of mineralizing phytic acid was isolated and believed responsible for the mineralization of phytic acid. Other workers have previously demonstrated that *Aspergillus niger* (4, 10) produces phosphatase enzymes which mineralize organic phosphorus.

Little interpretation could be made on the redistribution of free iron in the soil core because of the apparent effect of phytic acid upon the free iron extraction. However, the presence of iron in the white material in the effluent confirmed the movement of some iron out of the soil.

Although gamma irradiation of the soil core probably killed all microorganisms, it did not completely eliminate the mineralization of organic phosphorus. It is suggested that phosphatases already present in the soil before irradiation, and perhaps intracellular phosphatases released from cells lysed by gamma rays, may have been responsible for the limited amount of mineralization after gamma irradiation.

Practical implications of this study suggest that there would be little downward movement or leaching of organic phosphorus compounds in soil. From a genesis standpoint the amount of movement is probably significant; from a pollution standpoint involving leaching to ground-water supplies the movement is insignificant.

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