# THE AMOUNT AND UTILIZATION OF ORGANIC PHOSPHORUS IN SOILS

by

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# INTRODUCTION

This study was made in an attempt to determine the organic phosphorus in soils and to determine its role in plant nutrition.

Phosphorus, which is essential for proper plant growth, goes through a cycle in soils. Pierre (13) described this cycle as a dynamic system where soil, plant, and microorganism play important roles. Upon utilization, the phosphorus is synthesized by plants or organisms to become part of complex organic compounds. At various stages of decomposition these organic materials, containing phosphorus compounds, become a part of the environment of growing plants. In Kansas, the organic content of many soils is relatively high, and this may prove to be an important source of phosphorus for plants.

Is the organic phosphorus mineralized before usage? Some investigators (16) have reason to believe that some organic phosphorus may be utilized in the organic state. The largest organic phosphorus fraction is believed mineralized before usage. Pierre (13) claims that investigators at Iowa have established organic phosphorus as active in plant nutrition and not merely a frozen asset. If this is the case, much of the phosphorus-supplying power of soils may be obtained from plant residues and green manure crops. Similar findings in Kansas might prove valuable from the standpoint of establishing definite soil management practices that would keep the organic content of soils relatively high. On the other hand, Pierre (13) pointed out that many soils high in organic phosphorus are deficient in available phosphorus. Pierre (13) explained that the nature of the soil might have much to do in fixing the organic phosphorus in insoluble forms as calcium and iron precipitates.

Whatever the answers to those questions, working with this problem may lead to a better understanding of the complex phosphorus cycle. This understanding may lead to the primary objective of this research: An estimation of the importance of organic phosphorus in soil fertility.

## REVIEW OF LITERATURE

In 1920, Schollenberger (17) found approximately one-third of the phosphorus in the surface soil of Ohio was in the organic form. Recent investigations at Iowa (12) revealed the range of phosphorus in the organic form was from 35 per cent in the plow-layer to 72 per cent in the A' horizons of various soils, but soils were found to contain as little as 8 ppm in the C horizon.

Pierre (13) explained that three general types of compounds--nucleic acid, phytin, and lecithin--make up the bulk of organic phosphorus in plants and probably soils. Nucleic acids are considered the dominant type of organic phosphorus compounds in soils, plants, and microorganisms (13). Phytin was isolated in soils by Dyer and Wrenshall (7). Soils in Hawaii showed no evidence of nucleic acid compounds, but inositol phosphates, which are decomposition products of phytin, were isolated (22). Even though nucleic acids are considered the most abundant organic phosphorus compound in soils due to their ever-present nature in plants, Bower (3) found 40 to 49 per cent of the total organic phosphorus as phytin and its derivatives. Wrenshall and Dyer (21) found small amounts of phospholipids in soils.

Most of the organic phosphorus is believed to be mineralized before it is utilized by plants. Agents which have been studied and have been given credit for mineralization are microorganisms, enzymes, and heat.

Microbial activity was long considered the only factor in the decomposition of organic materials. This is still considered the most important phase of decomposition occuring in soils. Chang (5) noted that the increases and decreases of organic phosphorus were accompanied by corresponding decreases and increases of inorganic phosphorus. He pointed out that microorganisms synthesized considerable amounts of inorganic phosphorus in the decomposition of organic matter. Microorganisms mineralized nucleic acid very quickly, but the mineralization of lecthin was very slow (11). Liming did not influence the speed with which microorganisms decomposed nucleic acid, but it did speed up the decomposition of phytin (11).

Organic phosphorus may be mineralized by the enzymes produced by microorganisms (13). Rogers (15) proved that some dephosphorylation of organic phosphorus is due to root enzymes. He found that nucleic acids were readily mineralized by root enzymes, but lecithin and phytin were not. What portion of the catalytic properties causing phosphorus mineralization is due to root enzymes, and what portion is due to microbial enzymes is not known.

Thompson and Black (18) have attempted to measure the possible influence of temperature on the mineralization of organic phosphorus. Toluene and non-toluene-treated soils at various temperatures showed marked increases in inorganic phosphorus with increases in temperature. Toluene-treated samples showed a faster increase in inorganic phosphorus, possibly due to the destruction of the microorganisms by toluene. According to Pierre (13), Bower found that, during a month's incubation at 35°C., the virgin soils released an average of 39 ppm of phosphorus from the organic form; while, under the same conditions, the cropped soils released 11 ppm.

Studies were made by various investigators to determine the forms in which organic phosphorus is utilized by plants and the effectiveness of organic phosphorus materials as sources of phosphorus. Rogers et al. (16) found phytin and lecithin in nutrient solutions were not mineralized before utilization. The uptake of phytin from solutions was rapid; lecithin uptake was slow. Recent work has been conducted with radioactive phosphorus in the organic state. Such studies by Fuller and Dean (8) indicated that green manure supplied 70 per cent as much phosphorus as superphosphate. McAulliffe et al. (9) found protein-bound phosphorus in manure was 20 to 30 per cent as efficient as superphosphate. White et al. (19) found green manure crops were comparable to $KH_2OP_4$ in radioactive phosphorus studies.

## EXPERIMENTAL METHODS

One of the purposes of this research was to determine the phosphorus-supplying power of green manure crops and organic phosphorus-containing compounds to corn and alfalfa, respectively. It was also the purpose of this experiment to determine the fraction of phosphorus found in organic forms in soils and to determine the amount of this organic phosphorus fraction utilized by plants.

The soils used for greenhouse studies were the Marshall and Colby silt loams. The Marshall silt loam was collected on the Agronomy Farm, Riley County, Kansas, as a representation of the eastern part of the state. This soil was in sod, and showed no evidence of ever being cultivated. It appeared high in organic matter. The Colby silt loam, a dry land soil from the west, is from Garden City, Finney County, Kansas, which appeared low in organic matter and was under cultivation. It was the purpose of this experiment to select a soil high in organic matter and a soil low in organic matter for study. The crops which were grown in the greenhouse and the green manure crops--alfalfa and sweet clover--are common on Kansas farms. Lecithin and nucleic acid, which have been found present in soils, were added to the two soils as sources of phosphorus.

## Crop Utilization of Phosphorus from Green Manures and Organic Phosphorus Compounds

Each soil was screened through large mesh screen wiring, air-dried, and mixed thoroughly. Four thousand gram samples of soil were used in each treatment. Each treatment consisted of four replications. The nucleic acid was added in solution by dissolving it in one per cent $NH_4OH$. The solution was changed to a pH of 7 with Hcl. Lecithin was dissolved in 95 per cent pure ethyl alcohol. The solutions were added with a pipette to the soils on heavy paper. Each soil and solution was thoroughly mixed by tabling. Green manure crops were chopped up and mixed with the soil by tabling.

On September 9, 1949, the alfalfa and corn were planted in the soils, which had been wetted down. Ten seeds of hybrid corn were planted per pot to a depth of one inch. For the alfalfa treatments approximately 50 Buffalo alfalfa seeds were planted per pot just below the surface of the soil. On September 15, 1949, the alfalfa had to be replanted due to damping off and due to the sterilizing influence of ethyl alcohol in the lecithin-treated pots. After the corn attained the height of two to four inches, the corn plants were thinned to four per pot. The number of alfalfa plants left per pot ranged between 40 and 50 in number.

Standardized plaster of Paris blocks were inserted in one of each of the replications. Conductivity readings of the plaster of Paris blocks were obtained occasionally to keep the water content of the various soils in the different treatments fairly consistent. This method of measuring the moisture content of soils was devised by Bouyoucos and Mick (2). The soil was wetted down until, at equilibrium, the plaster of Paris blocks had a conductivity bridge reading of 2000 ohms. This was accomplished before seeds were planted in the soil. At that reading the moisture of the soil is considered 50 per cent available to plants (2). The water content of the various replications was kept fairly close to the 50 per cent availability level at the beginning of the growing season by the usage of daily conductivity readings. After the plant growth became fairly luxuriant and transpiration was fairly rapid, the water was supplied to the plants when needed. Fallow pots were kept fairly close to a conductivity reading of 2000 ohms.

## Crop Utilization of Phosphorus from Native Soil Organic Matter

Colby and Marshall silt loams, which had been tabled and mixed, were used for Neubauer seedling tests (6). Each treatment consisted of 40 grams of air-dry soil.

In order to study the availability of native soil organic phosphorus, one treatment consisted of soils deprived of their exchangeable and acid soluble phosphorus. This phosphorus fraction was extracted with a solution consisting of 0.03 normal $NH_4F$ and 0.1 normal Hcl. This extracting solution is the one used by Bray and Kurtz (4). Each replication was divided into two 20-gram samples for ease of extraction. The samples were extracted three times with 200 ml of extracting solution to insure the extraction of this fraction of phosphorus. The procedures involved in extracting this phosphorus fraction were to treat the soil with the extracting solution by shaking in an end-over-end shaker for 30 minutes, centrifuging, and pouring the extract into a container for phosphorus determination. This procedure was repeated thrice with the extracting solution and one time with water to remove the excess extracting solution. The three extracts obtained with the extracting solution were combined for phosphorus determination.

Another treatment of samples of Colby and Marshall silt loams was treated with $H_2O_2$ to mineralize the organic phosphorus. It was hoped that this treatment would provide a means of studying plant growth in the absence of organic phosphorus. Forty-gram samples were treated with 160 ml of 30 per cent $H_2O_2$. Samples were heated to dryness with 30 per cent $H_2O_2$. This was repeated three times to insure the mineralization of all the organic matter. Before the Colby was heated with $H_2O_2$, it was essential to make the calcareous soil acid as $H_2O_2$ is not an efficient oxidizing agent in alkaline solutions.

The other treatment consisted of the addition of an amount of inorganic phosphorus to the soil equivalent to the organic phosphorus present. This treatment provides a check against the $H_2O_2$ treatment since the two treatments differ only in that one contains native soil organic phosphorus and the other does not. The amounts of organic phosphorus in the soils were determined by Pearson's method (10). All samples were mixed with 50 grams of Hcl-washed sand and with necessary $CaCo_3$ to convert the soil at equilibrium to a pH of 7, as indicated by Tables 1 and 2. The Colby and Marshall silt loams that were deprived of available and acid soluble phosphorus and treated with $H_2O_2$ had their pH lowered due to the leaching of bases. Calcareous soils such as the Colby silt loam, when exposed to such treatments, lose their $CaCo_3$ content due to the presence of an acid solution and leaching. The Colby was limed with $CaCo_3$ to attain a pH of 7 at equilibrium, and enough $CaCo_3$ was added to convert the Colby to its original calcareous nature.

Table 1. Lime requirements of treated Marshall silt loam samples as determined by means of Woodruff's buffer solution.

| Treatments | PH after treatment | PH after treatment with Woodruff's buffered solution* | Lime requirement in pound per acre* |
|---|---|---|---|
| Phosphorus extracted | 3.08 | 5.55 | 14,500 |
| Inorganic phosphorus added | 5.45 | 6.64 | 4,600 |
| $H_2O_2$ treated to mineralize organic phosphorus | 5.37 | 6.67 | 3,300 |

* One thousand pounds of lime are added for each 0.1 drop in pH below a pH of 7.

Table 2. Lime requirements of treated Colby silt loam samples as determined by means of Woodruff's buffer solution.

| Treatments | PH after treatment | PH after treatment with Woodruff's buffered solution* | Lime requirement in pound per acre* |
|---|---|---|---|
| Phosphorus extracted | 3.27 | 5.92 | 10,800 |
| Inorganic phosphorus added | 7.76 | -- | -- |
| $H_2O_2$ treated to mineralize organic phosphorus | 3.10 | 5.10 | 19,000 |

* One thousand pounds of lime are added for each 0.1 drop in pH below a pH of 7.

The $CaCo_3$ necessary to change the soil at equilibrium to a pH of 7 was determined by Woodruff's buffer solution (20) as indicated in Tables 1 and 2. The $CaCo_3$ content in the Colby silt loam was determined by using Hutchinson and MacLennan's method as described by Piper (14). The Colby silt loam had an equivalence of 30,000 pounds of $CaCo_3$ per acre. Each sample, which consisted of 40 grams of soil, Colby or Marshall, and 50 grams of acid-washed silica sand thoroughly mixed, was placed into a small plastic dish and wetted down. One hundred rye seeds were planted November 16, 1949, into each dish so that each seed was half enclosed by the moist mixture, and the other half was exposed to the atmosphere. Rye was used due to its highly sensitive nature to respond to various stimuli. An additional 100 grams of acid-washed silica sand was added to each sample over the partly covered rye seedlings. All samples were brought to the same weight with water. During the growing season the weights of the samples were kept constant by the frequent additions of water. Seedlings were kept in the greenhouse and were protected from direct sunlight by using cheesecloth for a covering. Evidently large amounts of soluble salts were brought into the soil solution when the Colby soil was treated with $H_2O_2$. The salt concentration was so great in this treatment that the rye seeds failed to germinate. This particular treatment of the Colby soil was leached a number of times with water to remove excessive salts, and was replanted November 11, 1949. All samples were treated with the 200 ppm treatments of potassium and nitrogen. Seedlings were allowed to grow 17 days.

## Methods of Laboratory Analyses for Determining Phosphorus Content of Soils and Plant Materials

The two best-known methods of determining organic phosphorus in soils are Pearson's method (10) and the method of Bray and Kurtz (4). Most of the work in this research was made with Pearson's method (10). In both methods of analysis the dilutions used were slightly modified, and the reducing agent of Arnold and Kurtz (1), amino-naphthol-sulfonic acid, was used instead of stannous chloride.

One of the modifications from the recommended dilution of Pearson's method of analysis consisted of bringing the acid extraction of the bases to a volume of 50 ml instead of the recommended 200 ml. Two hundred ml of 1 normal $NH_4OH$ were used to extract phosphorus instead of the recommended 400 ml of 0.5 normal $NH_4OH$ (10). A five-ml aliquot of the acid extract and a 20-ml aliquot of the $NH_4OH$ extract were used to determine the organic phosphorus in soils. This decrease in dilution increased the accuracy of the method because it allowed for the measurement of greater concentrations of phosphorus.

The method of Bray and Kurtz (4) was slightly modified when the extraction of the organic phosphorus was brought to volume in a 200-ml volumetric flask.

Phosphorus contents of plants were determined by wet digestion with sulfuric, nitric, and perchloric acids (14). A one-gram sample of plant material was used for analysis. The final product of digestion was diluted to 100 ml. A two-ml aliquot was diluted to 50 ml for the determination of phosphorus.

All determinations, whether of plants or soils, were made of 20 ml of the final dilution in standardized colorimetric tubes. The method used to develop the color was the one given by Arnold and Kurtz (1), in which one ml of ammonium molybdate, $Hcl-H_3BO_3$, solution and one ml of the reducing agent, amino-naphthol-sulfonic acid, are used. Colorimetric determinations were made with the Evelyn Photometer. The ppm of phosphorus in the solutions tested were obtained from a standard curve. A standard curve was constructed by plotting known concentrations of phosphorus against the galvometer readings of the solutions. Little differences were found between the galvometer readings of solutions of known concentrations of phosphorus when the solutions contained the reagents used by Bray and Kurtz (4) and those by Pearson (10). The readings obtained are listed in Table 3.

Table 3. Average galvometer readings obtained for standard phosphorus solutions containing reagents used by Bray and Pearson.

| Concentration of solution in ppm of phosphorus | Galvometer readings using Bray's reagents | Galvometer readings using Pearson's reagents |
|---|---|---|
| 0.5 | 79[1] | 79 |
| 1.0 | 62[2] | 62[1] |
| 1.5 | 49[2] | 48[3] |
| 2.0 | 38[2] | 38[2] |
| 2.5 | 29[2] | 30 |
| 3.0 | 23[3] | 23[2] |
| 3.5 | 18[2] | 18[3] |
| 4.0 | 14[2] | 14[3] |

## EXPERIMENTAL RESULTS AND DISCUSSION

### Crop Utilization of Phosphorus from Green Manures and Organic Phosphorus Compounds

The corn was harvested November 14, 1949, and placed in the oven to dry. Corn plants were weighed and ground in preparation for determining their phosphorus contents. The purpose of this analysis was to determine the phosphorus-supplying power to corn of green manure crops.

The total uptake of phosphorus by the corn plants in the Marshall silt loam as indicated by Table 4 was not significantly different between treatments as statistically analyzed by the analysis of variance. Corn grown in the non-treated soil showed a greater percentage of phosphorus than the corn grown in the green manure-treated pots when statistically analyzed.

Table 4. Yield, total phosphorus uptake, and percentage of phosphorus of corn grown on Marshall silt loam with and without green manure treatment.

| Treatments | Rate of application: Pounds of dry material added per acre | Rate of application: Phosphorus added in the green manure Pounds per acre | Yield of corn Grams per pot* | Total phosphorus utilized Mgs. per pot* | Percentage phosphorus in corn* |
|---|---|---|---|---|---|
| No treatment | None | None | 21.6 | 19.66 | .091** |
| Alfalfa as green manure | 4,000 | 10.6 | 29.38*** | 20.27 | .069 |
| Sweet clover as green manure | 4,000 | 10.6 | 28.80*** | 20.22 | .070 |

* Averages of four replicate treatments.

** Differs significantly from the treated pots at the five per cent level. F = 5.14. L.S.D. (.05) = .017.

*** Differs significantly from non-treated pots at the one per cent level. F = 49.32. L.S.D. (.01) = 3.10.

Green manure treatments as indicated by the differences in the percentage phosphorus in corn in Table 4 seem to increase the need for phosphorus due to the increased supply of nitrogen. Green manure treatments in the Marshall silt loam increased yields significantly. It seems logical to conclude that nitrogen supplied by the green manure was responsible for increased yields.

The corn plants grown in the Colby silt loam failed to show significant responsive differences statistically as indicated by Table 5.

Table 5. Yield, total phosphorus uptake, and percentage of phosphorus of corn grown in Colby silt loam with and without green manure treatment.

| Treatments | Rate of application: Pounds of dry material added per acre | Phosphorus added in the green manure Pounds per acre | Yield of corn Grams per pot# | Total phosphorus utilized Mgs. per pot# | Percentage phosphorus in corn# |
|---|---|---|---|---|---|
| No treatment | None | None | 27.25 | 21.09 | .078 |
| Alfalfa as green manure | 4,000 | 10.6 | 26.75 | 23.92 | .088 |
| Sweet clover as green manure | 4,000 | 10.6 | 28.60 | 23.94 | .086 |

# Averages of four replicate treatments.

It is evident that such treatments of green manure crops failed to supply sufficient phosphorus to influence its uptake.

On December 9, 1949, the alfalfa crop was harvested and placed in the oven to dry. Only one alfalfa cutting was obtained. Each sample was weighed and ground in preparation for determining the phosphorus content of the alfalfa in the variously treated pots. It was the purpose of this experiment to determine the phosphorus-supplying power to alfalfa of organic compounds that have been found present in soils. The two compounds used were lecithin (animal) and nucleic acid (yeast) from Eastman Kodak Company.

Results on the Marshall silt loam showed no significant differences statistically between treatments in regard to the total phosphorus utilized by alfalfa and the phosphorus percentage of the alfalfa as indicated by Table 6. Nucleic acid increased the yield of alfalfa significantly over lecithin.

Table 6. Yield, total phosphorus uptake, and percentage of phosphorus of alfalfa grown on Marshall silt loam with and without organic phosphorus materials added.

| Treatments | Rate of application: Pounds of material added per acre | Rate of application: Phosphorus added in the phosphorus materials Pounds per acre | Yield of alfalfa in grams per pot* | Total phosphorus utilized Mgs. per pot* | Percentage phosphorus in alfalfa* |
|---|---|---|---|---|---|
| No treatment | None | None | 2.31 | 6.09 | .27 |
| Lecithin | 1,650 | 50 | 2.06 | 6.62 | .33 |
| Nucleic acid | 650 | 50 | 2.61** | 10.41 | .38 |

* Averages of four replicate treatments.

** Differs significantly from other treatments at the five per cent level. F = 5.88. L.S.D. (.05) = .36.

The nucleic acid treatments in the Colby silt loam increased the uptake of phosphorus by alfalfa significantly at the five per cent level as indicated in Table 7. Nucleic acid also increased the percentage of phosphorus in the alfalfa plants. Yields were not increased significantly by the treatments.

Table 7. Yield, total phosphorus uptake, and percentage of phosphorus of alfalfa grown on Colby silt loam with and without organic phosphorus materials added.

| Treatments | Rate of application: Pounds of material added per acre | Rate of application: Phosphorus added in the phosphorus materials: Pounds per acre | Yield of alfalfa in grams per pot* | Total phosphorus utilized Mgs. per pot* | Percentage phosphorus in alfalfa* |
|---|---|---|---|---|---|
| No treatment | None | None | 2.75 | 6.40 | .24 |
| Lecithin | 1,650 | 50 | 2.76 | 7.59 | .30 |
| Nucleic acid | 650 | 50 | 2.94 | 8.56** | .29** |

* Averages of four replicate treatments.

** Differs significantly from no treatment pots at the five per cent level. $F = 11.89$. Significance was obtained only when no treatment and nucleic acid were statistically analyzed separately from lecithin.

Lecithin-treated pots failed to show any differences statistically over the nontreated pots.

Alfalfa utilized a greater amount of phosphorus when treated with nucleic acid. Nucleic acid appears to be an active source of phosphorus in soils. The increased yields produced by nucleic acid over lecithin in the Marshall silt loam indicates that it may be more easily available than lecithin. The utilization of phosphorus by alfalfa was significantly greater in the Colby silt loam treated with nucleic acid than the Colby receiving no treatment. Failure to obtain increased yields indicates that, in the Colby, phosphorus is not a limiting factor. Lecithin-treated samples demonstrated unusual variability due probably to the retarding influence of ethyl alcohol, which reacts as a sterilizing agent. Erratic results were obtained with the alfalfa crop. Erratic results are often obtained with first alfalfa cuttings, which was the only cutting obtained, due partially to uneven distribution of the alfalfa roots in the pots.

### Crop Utilization of Phosphorus from Native Soil Organic Matter

On December 3, 1949, all rye seedlings plus roots, except the soil containing inorganic phosphorus only, were harvested and placed in the oven to dry. The roots were separated from the soil and sand mixture with a strong stream of water. Each sample was weighed and ground in preparation for the determination of its phosphorus content. Only two replicates grown in the soil containing only inorganic phosphorus germinated, and these were harvested December 21, 1949, for analysis as previously described. Analyses of the various treatments were made in an attempt to determine the role soil organic phosphorus plays in plant nutrition.

Treatments used in this experiment with the Marshall silt loam and results obtained are given in Table 8.

Table 8. Yield, total phosphorus uptake, and percentage phosphorus of rye grown on Marshall silt loam with various treatments by the Neubauer seedling method.

| Forms of phosphorus desired in soil | Treatments given soil in attempt to attain desired phosphorus forms | Rate of application of the fertilizer elements Lbs. per acre N | K | P | Amount of phosphorus extracted Lbs. per acre* | Yield of rye Gms. per pot* | Total phosphorus utilized Mgs. per pot* | Percentage phosphorus in corn* |
|---|---|---|---|---|---|---|---|---|
| Organic phosphorus only | Phosphorus extracted | 200 | 200 | None | 139.8 | 1.39 | 7.61 | .55 |
| Inorganic phosphorus and organic phosphorus | Inorganic phosphorus added | 200 | 200 | 350 | None | 1.60 | 10.58** | .66 |
| Inorganic phosphorus only | $H_2O_2$ treated to mineralize organic phosphorus | 200 | 200 | None | None | 1.35 | 7.95 | .60 |

*Averages of four replicates treatments.

**Differs significantly from extracted phosphorus and $H_2O_2$ treatment at the one per cent level. F = 54.66. L.S.D. (.01) = 1.15. L.S.D. (.05) = .76.

When the soil contained inorganic phosphorus plus organic phosphorus, markedly more phosphorus was utilized by the plants than when the soil contained a similar amount of inorganic phosphorus only. There was no significant differences in the amount of phosphorus utilized when the soil contained inorganic phosphorus only and when it contained organic phosphorus only.

These results indicate that organic and inorganic phosphorus supplies in the Marshall silt loam are of about equal availability to plants. This would indicate that the equivalent of 190 ppm of organic phosphorus in the Marshall silt loam was utilized by plants. Other sources of phosphorus must be contributing phosphorus to the plants as this is a larger organic phosphorus content than found in this soil by Pearson's method. The rye grown on the soil containing inorganic phosphorus plus organic utilized an average of 66 ppm of phosphorus more than rye grown on a soil containing a similar amount of inorganic phosphorus only. This would indicate that an average of 38 per cent of the organic phosphorus as determined by Pearson's method was utilized by rye in the Marshall silt loam.

There were no significant differences between treatments in the phosphorus percentage of the rye and in the yield of the rye grown on the Marshall silt loam. The main difficulty encountered in this respect was the variability within the same treatment, especially in the case of the soils containing inorganic phosphorus only and organic phosphorus only. A possible explanation for such an occurrence was the influence of chemical treatment on the physical condition of the soil. Roots developed much more favorably in the soil containing both inorganic and organic phosphorus. A reduction in the available inorganic phosphorus in Table 8 may result in the greater usage of organic phosphorus.

No statistical analyses were made of the Neubauer seedling test on the Colby silt loam, as only two of the replicates containing inorganic phosphorus alone germinated. Results of this experiment are given in Table 9. The phosphorus uptake of rye in the Colby silt loam containing organic phosphorus only was equivalent to 202 ppm. This again indicates that some inorganic phosphorus, which was not extracted, is supplying phosphorus to the rye plants. The rye grown on the soil containing inorganic plus organic phosphorus utilized an average of 16 ppm of phosphorus more than rye grown on a soil containing a similar amount of inorganic phosphorus only. This would indicate that an average of 24 per cent of the organic phosphorus, as determined by Pearson's method (10), was utilized by rye in the Colby silt loam.

The physical condition of the Colby silt loam was disrupted similar to the Marshall silt loam. Salt concentration accumulated in the Colby silt loam treated with $H_2O_2$, and leaching was essential before germination occured.

The averages in Table 9 indicate that the organic phosphorus fraction in the Colby silt loam is not as important as in the Marshall silt loam. This is shown by a greater response in organic phosphorus absorption, 66 ppm, in the Marshall silt loam as compared to 16 ppm in the Colby silt loam. The results of the Neubauer seedling tests indicate that the native soil organic phosphorus is active in plant nutrition.

Table 9. Yield, total phosphorus uptake, and percentage phosphorus of rye grown on Colby silt loam with various treatments by the Neubauer seedling method.**

| Forms of phosphorus desired in soil | Treatments given soil in attempt to attain desired phosphorus forms | Rate of application of the fertilizer elements Lbs. per acre | | | Amount of phosphorus extracted Lbs. per acre* | Yield of rye Gms. per pot* | Total phosphorus utilized Mgs. per pot* | Percentage phosphorus in corn* |
|---|---|---|---|---|---|---|---|---|
| | | N | K | P | | | | |
| Organic phosphorus only | Phosphorus extracted | 200 | 200 | None | 939 | 2.19 | 8.08 | .37 |
| Inorganic phosphorus and organic phosphorus | Inorganic phosphorus added | 200 | 200 | 130 | None | 1.89 | 9.80 | .52 |
| Inorganic phosphorus only | $H_2O_2$ treated to mineralize organic phosphorus | 200 | 200 | None | None | 2.35 | 9.15 | .39 |

* Averages of four replicate treatments with the exception of $H_2O_2$ which consisted of two replications.

** No statistical analysis was made due to the loss of two replications of the $H_2O_2$ treatments.

## Method of Laboratory Analyses for Determining Phosphorus Contents of Soils and Plant Materials

It was the purpose of this research to attempt to determine the organic phosphorus of the various treatments after crops had been grown on them. Determinations of Pearson's method (10) and the method of Bray and Kurtz (4) showed the necessity of a better method of analyzing for this fraction of phosphorus. Both methods failed to give sufficient accuracy for such a study.

Much of the work in this study was spent in an effort to improve the accuracy of Pearson's method (10) for analyzing organic phosphorus. Pearson's method consists of extracting the bases with 0.1 normal Hcl in the preliminary extraction. The organic phosphorus fraction is extracted with 0.5 normal $NH_4OH$ at 89° to 92° C. for 16 to 18 hours. Proportionate aliquots of the two extracts are combined to determine the inorganic and the total phosphorus in solution. The organic phosphorus is determined by difference. Attempts were made to determine the factor or factors which often led to erratic results and required many analyses. Dilutions were reduced to improve the accuracy of this method as previously indicated. The recommendations by Pearson (10) concerning certain factors--temperature, dilution, extraction of the bases, normality of the extracting solution, and the length of digestion--were followed. The changes made in the dilution and in the normality of the extracting solution were not considered critical by Pearson in his study. Evidently other factors were causing erratic results. Theoretical contributing factors may be listed as: (1) the heterogeneous system of the soil, (2) the hydrolysis of the organic phosphorus by heat during digestion, (3) the fixation of the extracted phosphorus, and (4) the errors involved in determinations by differences.

Thompson and Black (18) found that a large amount of organic phosphorus is mineralized at 90° C. when incubated for one week. They pointed out that the organic phosphorus may be hydrolyzed to inorganic phosphorus during Pearson's digestion procedure. Thompson and Black (18) indicated that Schmoeger found when peat soils were heated in an autoclave at 130° to 160° C. for 20 hours, the quantity of phosphorus extracted with Hcl was the same as the quantity that could be extracted from the ash of the same soil. Thompson and Black showed that, in every case, the inorganic phosphorus extracted by 1 normal $H_2SO_4$ was less than the inorganic phosphorus extracted by the extracting solution, 0.5 normal $NH_4OH$ as used by Pearson (10). The increase in inorganic phosphorus ranged from 50 to 100 ppm. These facts indicate that a temperature of 89° to 92° C. used in Pearson's method (10) increase the hydrolysis of easily decomposable organic phosphorus.

The extracted phosphorus may be fixed by clay minerals and calcium compounds that were placed in solution by the digestion procedure, and by flocculation of the more complex organic phosphorus compounds by $NH_4Cl$. According to Pierre (13), Bower found the amount of nucleic acid was reduced from 91 to 16 per cent in a four-day period by montmorillonite. Increased temperatures have a tendency to render most minerals more soluble. It is logical to assume that the amount of calcium compounds in solution would increase with temperature. Upon cooling, these compounds could react with inorganic phosphorus to form precipitates. Phytin (13) showed evidence of forming insoluble calcium and ferric salts. These organic phosphorus compounds could precipitate with calcium when the samples were allowed to cool off in the alkaline extract. It was pointed out by Bower (3) that 40 to 49 per cent of the total organic phosphorus in some soils is in the form of phytin and its derivatives.

Ammonium chloride in flocculating the soil, though Pearson (10) claimed it did not flocculate nucleic acid, may fix some of the complex organic phosphorus compounds such as phytin.

Any error is often emphasized when the determination is made by differences.

The average amounts of organic phosphorus found by Pearson's method (10) in soils are listed in Table 10. The percentage of organic phosphorus in the extract refers to the fraction organic phosphorus is of the total phosphorus extracted by Pearson's method.

Table 10. The average amounts of organic phosphorus in some Kansas soils and the average percentages of organic phosphorus in the soil extract as determined by Pearson's method (10).

| Soil | Amount of organic phosphorus in soil ppm | Percentage organic phosphorus in extract |
|---|---|---|
| Geary silt loam | 154.5 | 42.14 |
| Lamoure clay | 145.8 | 30.78 |
| Marshall silt loam | 174.9 | 34.86 |
| Colby silt loam | 66.4 | 7.31 |
| Cherokee silt loam | 59.8 | 13.47 |
| Munjor silty clay loam | 63.9 | 6.09 |
| Uncultivated Chernozem 0-4" | 222.9 | 30.27 |
| Uncultivated Chernozem 0-12" | 195.1 | 27.09 |

The first three soils were collected on the Agronomy Farm, Riley County, Kansas. The Lamoure clay was under cultivation. The Geary and Marshall silt loams were in sod. The Munjor soil is from Hays, Kansas; the Colby silt loam from Garden City, Kansas. The Cherokee silt loam is from Cherokee County, Kansas. The Chernozem samples were obtained from an uncultivated native grass pasture in the Chernozem belt near Hays, Kansas.

In an attempt to determine the organic phosphorus utilized by the crops in the greenhouse, determinations of the organic phosphorus were made of the various treatments after the crops had been grown. The method devised by Bray and Kurtz (4) was used for this purpose. The inorganic phosphorus in Bray and Kurtz's method is extracted with 0.5 normal $NH_4F$-0.1 normal Hcl solution. The organic phosphorus is mineralized with 30 per cent $H_2O_2$, and it is extracted like the inorganic as described above. The organic phosphorus is determined by difference. The soils determined are listed below in Table 11. Here again the accuracy of the method failed to serve the purpose. When the soils were collected for studies, a sample was collected for the determination of phosphorus. Fallowed soils were kept in the greenhouse and watered with the treatments grown to corn. Soils which received no treatment were not treated to green manures, and planted to corn. Alfalfa and sweet clover were the green manure crops added to the soils before the corn was planted.

Table 11. Average amounts of organic phosphorus determined by Bray and Kurtz's method (4) in soils from the field and after use in greenhouse experiments.

| Treatments | Soils: Marshall silt loam: organic phosphorus in ppm 4/ | Soils: Colby silt loam: organic phosphorus in ppm 4/ |
|---|---|---|
| Field conditions: | | |
| Soil as collected in the field 1/ | 466.9 | 443.8 |
| Greenhouse conditions: | | |
| Fallow 3/ | 510.7 | 369.4 |
| No treatment 2/ | 481.4 | 390.7 |
| Sweet clover green manure 2/ | 527.3 | 384.8 |
| Alfalfa green manure 2/ | 447.9 | 416.9 |

1/ Soil as collected in the field for studies.
2/ Organic phosphorus determination for soil made after corn growth.
3/ Fallow treatments, which were watered, were analyzed for organic phosphorus with the other greenhouse treatments.
4/ Each treatment consisted of four replications.

The high results given in Table 11 seem to indicate that some mineral phosphorus is brought into solution by the vigorous reaction of $H_2O_2$. The averages indicate the impossibility of determining any changes in organic phosphorus due to crop growth. The differences within treatments were just as great as the differences between treatments.

## SUMMARY

It was the purpose of this research to determine the amount of organic phosphorus in Kansas soils and to determine the role played by organic phosphorus in soil fertility. Much work remains to be done before these objectives can be fully achieved.

Additional organic phosphorus materials were added to soils under greenhouse conditions in an attempt to determine the role of organic phosphorus in soil fertility. The organic phosphorus containing materials added were nucleic acid, lecithin, and green manures. The soils used in greenhouse studies were Marshall silt loam from Riley County, Kansas, and Colby silt loam from Finney County, Kansas. Green manure treatments were used in an attempt to determine the value of these crops to Kansas farmers as sources of phosphorus. Lecithin and nucleic acid were used as organic phosphorus treatments due to their occurrence in soils.

Corn grown in green manure-treated soils failed to utilize more phosphorus than corn grown without green manure. Green manure treatments increased the yield of corn over no treatment in the Marshall silt loam. The percentage phosphorus in the corn of the untreated Marshall silt loam was greater than in the treated soils. No differences in responses were obtained between treated and untreated Colby silt loam. Nitrogen proved to be the element that increased the yield of the treated Marshall silt loam. Green manure treatments definitely failed to supply enough organic phosphorus to increase the utilization of phosphorus by corn in the soils studied. The application of nitrogen in the form of green manure crops possibly increased the needs for phosphorus fertilization in the Marshall silt loam as indicated by a lower percentage phosphorus in the corn grown on the treated soils.

Treatment of the Marshall silt loam with nucleic acid and lecithin did not increase significantly the total amount of phosphorus utilized or the percentage of phosphorus in the alfalfa plants. In this respect the average values obtained for the nucleic acid and lecithin-treated soils were greater than the untreated soils. The variability within treatments may have prevented significant differences from being obtained. Nucleic acid treatments did increase the yield of alfalfa over lecithin treatments and approached the significance level over untreated soils.

Nucleic acid additions to Colby silt loam increased significantly the total phosphorus utilized by alfalfa and the percentage phosphorus in the alfalfa. There was no difference in yield between the treatments.

The increased yields of alfalfa, greater phosphorus utilization by alfalfa, and percentage phosphorus in alfalfa due to nucleic acid treatments in the Marshall silt loam indicate that nucleic acid is an important source of phosphorus in soils. Significant increases of total phosphorus removed and percentage phosphorus in the alfalfa grown on nucleic acid-treated Colby silt loam indicated that it does contribute available phosphorus for plants. Its failure to increase yields in the Colby silt loam indicated that phosphorus is not a limiting factor in this soil.

The averages of total phosphorus utilized and percentage phosphorus utilized by alfalfa in the lecithin-treated soils are greater than in the untreated soils. Lack of significant differences due to this treatment over untreated soils was partially due to the retarding influence of ethyl alcohol on alfalfa. Ethyl alcohol was used as a solvent. The averages indicate that it is a source of phosphorus in soils.

Neubauer seedling tests were used in an attempt to determine the role native soil organic phosphorus plays in plant nutrition. Rye grown on Marshall silt loam containing inorganic plus organic phosphorus utilized 66 ppm more phosphorus than rye on the same soil containing a similar amount of inorganic phosphorus only. Rye grown on the Marshall silt loam which contained only inorganic phosphorus failed to utilize more phosphorus than the rye grown on the soil which contained organic phosphorus only. Rye grown on soil containing only organic phosphorus, a soil deprived of extractable and acid soluble phosphorus, utilized the equivalent of 190 ppm of phosphorus, which is higher than the amount of organic phosphorus in this soil as determined by Pearson's method. The rye must have obtained some of its phosphorus from other sources besides organic. In both cases, though, the native soil organic phosphorus of the Marshall silt loam appeared active in plant nutrition.

Average results obtained with the Colby silt loam, though not statistically analyzed due to sample losses, appeared similar to the Marshall silt loam. Indications are that organic phosphorus is not as important a source of phosphorus in the Colby silt loam as in the Marshall silt loam. Average results obtained showed that the rye grown on Colby silt loam containing only inorganic phosphorus utilized more phosphorus than the rye grown on the Colby containing only organic phosphorus. The high utilization of phosphorus in this soil, as in the Marshall silt loam, indicated that the Colby containing organic phosphorus only was obtaining its phosphorus from other sources besides organic, possibly tightly adsorbed, which was not extracted. The results also showed that the rye grown on the Colby containing inorganic phosphorus plus organic utilized more phosphorus, the equivalent of 16 ppm, than the rye grown on the Colby containing a similar amount of inorganic phosphorus only.

The results obtained with the Neubauer test indicated that native soil organic phosphorus is important in plant nutrition. Rye utilized, on the average, an additional 66 ppm of phosphorus in the Marshall silt loam when an increment of organic phosphorus was present. This represents 38 per cent of the organic phosphorus present in the Marshall silt loam as determined by Pearson's method. Average increase of phosphorus utilized by rye in the Colby silt loam was 16 ppm when an increment of organic phosphorus was present. This represents 24 per cent of the organic phosphorus in this soil as determined by Pearson's method. This indicates that the organic phosphorus plays a greater role in the Marshall silt loam than in the Colby silt loam.

The methods used for determining organic phosphorus in soils are the ones used by Pearson (10) and Bray and Kurtz (4). Both methods failed to demonstrate sufficient accuracy for determining the changes in the organic phosphorus content of soils after crop growth. Pearson's method was the method used most extensively. The inaccuracy of Pearson's method might be due to the fixation of phosphorus by soil factors after the extraction, which is made in $NH_4OH$ at 89° to 92° C. Calcium, which is brought in solution by this increased temperature, might form precipitates with phosphorus in alkaline solution when allowed to cool. Fixation by clay could occur as clay has the ability to fix organic phosphorus. Ammonium chloride, used to flocculate the soil suspension before filtering, could cause fixation of organic phosphorus. The two methods determine organic phosphorus by difference. Errors are often emphasized by this form of determination. Probably one of the greatest factors that contributes towards erratic results in Pearson's method is the hydrolysis of some of the easily decomposable organic phosphorus at 89° to 92° C.

The method of Bray and Kurtz was used only in a few determinations. The high organic phosphorus results obtained by this method indicated that the $H_2O_2$ reaction is too vigorous and brings mineral phosphorus into solution, which is calculated as organic phosphorus.

The average ppm of organic phosphorus in Kansas soils, as determined by Pearson's method, ranged from over 200 ppm in a virgin Chernozem soil of Kansas to slightly over 60 ppm in a sample of Munjor silty clay loam.

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LITERATURE CITED

(1) Arnold, C. V., and L. T. Kurtz.
Photometer method for determining available phosphorus in soils. Ill. Agr. Expt. Sta. Agron. Dept. Pamphlet AG 1306. 1946.

(2) Bouyoucos, G. J., and A. H. Mick.
An electrical resistance method for the continuous measurement of soil moisture under field conditions. Mich. Agr. Expt. Sta. Tech. Bul. 172. 1940.

(3) Bower, C. A.
Separation and identification of phytin and its derivatives from soils. Soil Sci. 59:277-285. 1945.

(4) Bray, R. H., and L. T. Kurtz.
Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39-45. 1945.

(5) Chang, S. D.
Phosphorus transformation during decomposition of plant material. Soil Sci. 48:85-99. 1939.

(6) Diagonostic techniques for soils and crops. Published by the American Potash Institute. H. B. Kitchen, editor. p. 210-215.

(7) Dyer, W. J., and C. L. Wrenshall.
Organic phosphorus in soils: I. The extraction and separation of organic phosphorus from soils. Soil Sci. 51:159-170. 1941.

(8) Fuller, W. H., and L. A. Dean.
Utilization of phosphorus from green manures. Soil Sci. 68:197-202. 1949.

(9) McAulliffe, C., M. Peech, and R. Bradfield.
Utilization by plants of phosphorus in farm manure: II. Availability to plants of organic and inorganic forms of phosphorus in sheep manure. Soil Sci. 68:185-195. 1949.

(10) Pearson, R. W.
Determination of organic phosphorus in soils. Indus. and Engg. Chem. Analyt. Ed. 12:198-200. 1940.

(11) Pearson, R. W., A. G. Norman, and C. Ho.
The mineralization of organic phosphorus of various compounds in soils. Soil Sci. Soc. Amer. Proc. 6:168-175. 1941.

(12) Pearson, R. W., and R. W. Simonson.
Organic phosphorus in seven Iowa soil profiles: Distribution and amount as compared to organic carbon and nitrogen. Soil Sci. Soc. Amer. Proc. 4:162-167. 1939.

(13) Pierre, W. H.
The phosphorus cycle and soil fertility. Amer. Soc. Agron. Jour. 40:1-14. 1948.

(14) Piper, C. S.
Soil and plant analysis. New York: Inter-science Publishers. pp. 272-274 and pp. 130-132. 1944.

(15) Rogers, H. T.
Dephosphorylation of organic phosphorus compound by soil catalysts. Soil Sci. 54:439-446. 1942.

(16) Rogers, H. T., R. W. Pearson, and W. H. Pierre.
Adsorption of organic phosphorus by corn and tomatoes, and the mineralization action of exo-enzyme system of growing roots. Soil Sci. Soc. Amer. Proc. 5:285-291. 1940.

(17) Schollenberger, C. J.
Organic phosphorus content of Ohio soils. Soil Sci. 10:127-141. 1920.

(18) Thompson, L. M., and C. A. Black.
The effect of temperature on the mineralization of soil organic matter. Soil Sci. Soc. Amer. Proc. 12: 323-326. 1947.

(19) White, J. L., M. Fried, and A. J. Ohlrogge.
A study of the utilization of phosphorus in green manure crops by the succeeding crop, using radioactive phosphorus. Agr. Jour. 41:174-175. April, 1949.

(20) Woodruff, C. M.
Treating soils for lime requirement by means of a buffered solution and the glass electrode. Soil Sci. 66:53-63. 1948.

(21) Wrenshall, C. L., and W. J. Dyer.
Organic phosphorus in soils: II. The nature of the organic phosphorus compounds; A. Nucleic acid derivatives, B. Phytin. Soil Sci. 51:235-248. 1941.

(22) Yoshida, R. K.
Studies on organic phosphorus compounds in soils: Isolation of inositol. Soil Sci. 50:81-89. 1940.