

A STUDY OF ORGANIC PHOSPHORUS
IN OKLAHOMA SOILS

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INTRODUCTION

Early investigators discovered that part of the soil phosphorus was present in an organic form and that it may make up a high percentage of the total phosphorus in the soil. More recent investigation has shown that organic phosphorus in soil can be separated quantitatively from the inorganic phosphorus compounds. Methods have been developed to fractionate the organic phosphorus and such substances as nucleic acid, lecithin, inositol, and phytin have been isolated and identified.

From the thousands of tests that have been made to determine the availability of soil phosphorus by extraction with solutions of varying degree of acidity, it is clearly evident that many soils are very deficient in inorganic phosphorus; however, the availability of organic phosphorus cannot be determined by these methods. When weather conditions are favorable good crops are frequently produced on soils very low in available inorganic phosphorus but well supplied with organic matter from the addition of organic residue with a narrow carbon-nitrogen ratio.

This investigation has been planned to study the relation between organic phosphorus and total nitrogen in soil organic matter as affected by cultivation.

REVIEW OF THE LITERATURE

As early as 1844 Mulder (16) noted the presence of phosphorus in organic soil material.

Hopkins and Pettit (13) noted that certain soils showed a uniform mineral composition in surface, subsurface, and subsoil, and suggested that the difference in phosphorus in surface and subsoil might be due to organic phosphorus.

In 1904, Aso (1) identified lecithin, the first organic phosphorus compound isolated in soil.

Stewart (29) discussed the solubility of soil phosphorus in hydrochloric acid and ammonia and concluded that the greater part of the ammonia soluble phosphorus was organic.

Fraps (10) in 1911 showed that organic phosphates were soluble in ammonia after extraction with hydrochloric acid. He concluded that no method previously advocated would distinguish between organic and inorganic phosphorus in ammonia extracts.

Shorey (26) in his studies isolated nucleic acid in soil extracts.

Potter and Benton (23) originated a method for distinguishing between organic and inorganic phosphorus in soil extracts.

Schollenberger (24) developed further the method of Potter and Benton. He showed that certain relationships exist between organic phosphorus and other soil constituents. He concluded that organic phosphorus and nitrogen exist in the same ratio at different depths.

Auten (2) analyzed Iowa soils and gave calculations comparing the nitrogen-carbon, organic phosphorus-total phosphorus, organic phosphorus-organic carbon, and organic phosphorus-nitrogen ratios.

His results showed that the organic phosphorus-nitrogen ratio varied from 1-8 to 1-24 in the four soils tested. Organic phosphorus-total phosphorus varied from 1-3 to 1-20. In a later paper (3) he attempted to identify some of the organic compounds in soils in which phosphorus is combined. He concluded as follows:

"Very probably the organic phosphorus is present in soils as the calcium, magnesium, iron, or aluminum salts of the phosphorus, carbohydrate, amino acid complex and not absorbed from the phosphoric acid liberated by the hydrochloric acid from inorganic phosphorus salts. There are two possibilities at least, (a) that the organic phosphorus is formed under conditions existing when the hydrochloric acid liberates inorganic phosphorus from inorganic salts, or (b) that it exists as the calcium salt of the organic phosphorus complex which, when the calcium is removed, becomes soluble because of the formation of the ammonia salt. It is a well-known fact that without previous acid extraction ammonia will dissolve very little organic phosphoric acid; but after HCl extraction practically all of it is dissolved."

Auten disagrees with other investigators on the occurrence of large amounts of nucleic acid, phytin, or lecithin in soils. His experiments showed that when these compounds were incubated in silica sand for three months 85 percent of the nucleic acid phosphorus, 67 percent of the phytin phosphorus, and 66 percent of the lecithin phosphorus had hydrolyzed to the inorganic form.

Whiting and Heck (31) have shown remarkable success using phytin as a source of phosphate for growing oats and red clover.

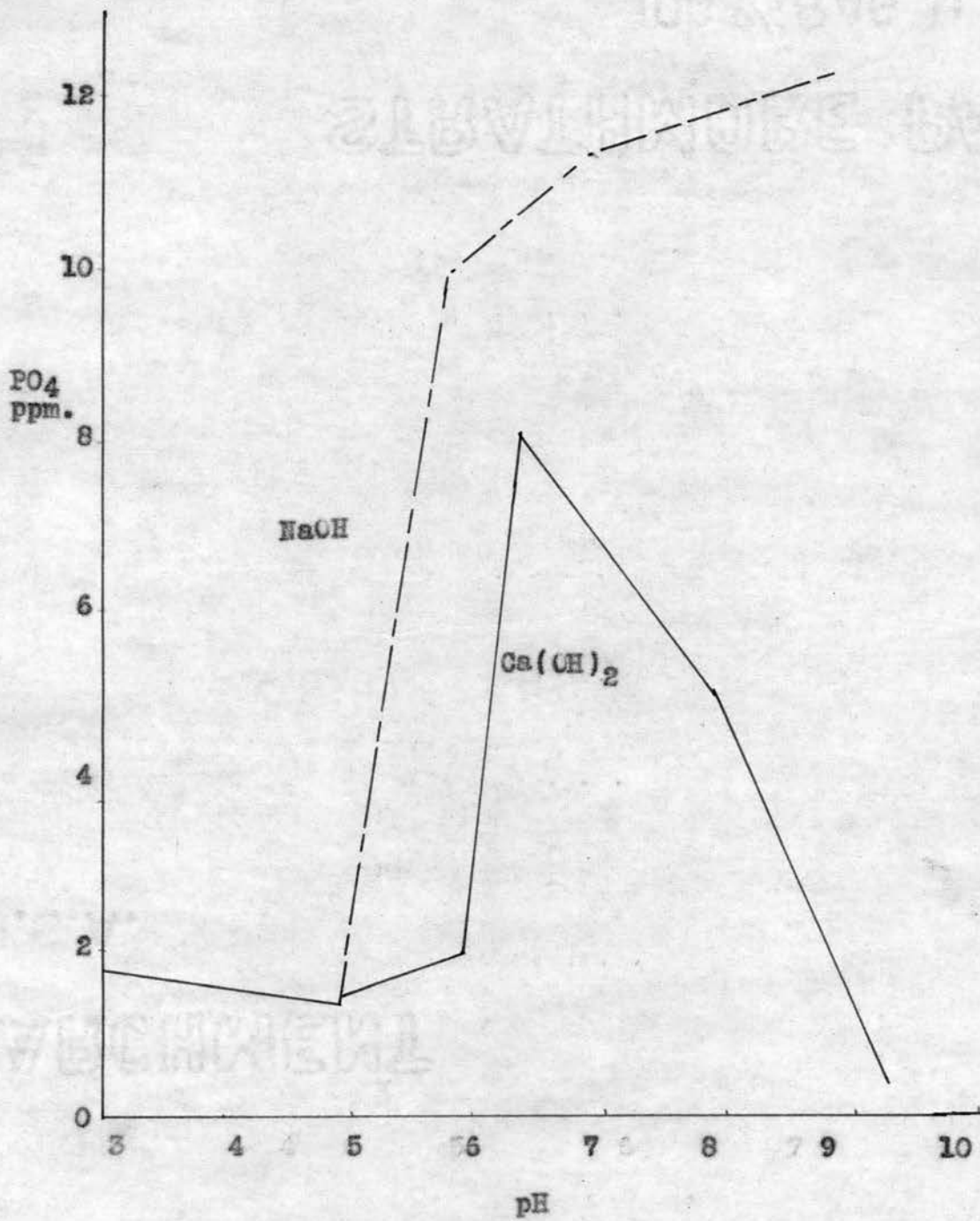
Pierre and Parker (21) on the other hand, found that plants grown in soil-solution cultures would not take up phosphates of the organic form.

McGeorge and Breazeale (15) in their studies with green and barnyard manures concluded that the organic phosphates in these types of fertilization were of considerable importance. They believed that of the organic phosphate compounds, the most important and abundant are phytin, which is a calcium-magnesium salt of inositol phosphoric acid,

lecithin, which is a choline-glycerol-phosphoric acid, and nucleic acid, the full composition of which is unknown, but is known to break down into phosphoric acid, a carbohydrate phosphoric acid, a carbohydrate and a purine, or pyrimidine base. Phytin is probably the most abundant in plant materials. Since they believed phytin to be the most abundant of the organic phosphorus compounds in soil, they checked the solubility of the pure compound and found it to be 9 p.p.m. in pure water and 11 p.p.m. in CO₂ saturated water. When the solubility was tested in solutions containing CaCO₃ and NaHCO₃, the solubility was further increased to 15.2 and 16.0 p.p.m., respectively. This indicates that phytin should be readily soluble in alkaline soils. Their studies also indicate that phytin reacts toward changes in pH in a manner similar to the iron and aluminum phosphates in that it is much less soluble in an acid solution than in an alkaline solution. Above pH 7.0, in NaOH, the solubility increases, while in solutions of Ca(OH)₂, solubility steadily decreases with the increase of pH until it is practically all precipitated at pH 9.0. Figure 1 shows their results regarding the effect of pH on the solubility of phosphate in phytin. Their findings agree with those of Auten in that phytin when incubated in either an aerated or puddled soil condition increased the solubility of PO₄.

Spencer and Stewart (28) studied the use of organic phosphates which might have a greater soil-penetrating power than the common inorganic phosphate carriers. Their results showed that phosphorus in organic phosphates of the type formula R(OH)₂(OPO₃R_y)₂ escaped, to a marked degree, the fixation which occurs to a phosphorus applied in some inorganic form. Specific examples of the organic phosphates they used are calcium-mono-orthophosphate of glycerol, C₃H₅ (OH)₂-OPO₃Ca, and potassium sorbityl di-orthophosphate, C₆H₈ (OH)₄-(OPO₃K₂)₂. Analysis of

FIGURE 1. EFFECT OF pH UPON THE SOLUBILITY OF THE PHOSPHATE IN PHYTIN.



the water solutions passing through the soil demonstrated that whereas 88-99 percent of the inorganic phosphate was fixed, only 5-20 percent of the organic compounds were retained by the soil.

Hilbert and others (12) made more extensive studies of the work done by Spencer and Stewart on the use of organic phosphates as fertilizers and on their ability to resist fixation. They found that the fixation of organic phosphate varies widely with the nature of the soil. In Cecil soil, which possessed about 40 percent colloidal matter, the organic phosphate was converted almost as rapidly and completely to an insoluble form as was inorganic phosphate. They found that the increase in fixation in "raw" soil is a secondary reaction following the primary action of soil microorganisms on the organic phosphate.

Odynsky (18) used an ignition method to determine the amount of organic phosphorus in layers at different depths in five Alberta soils. Amounts as high as 1,105 p.p.m. were found in the surface layers, whereas the deepest samples contained 41 to 91 p.p.m.

Dean (6) separated the organic from the inorganic phosphorus in the soil by digesting it overnight at 95° C. with (1-20 extraction) 0.25 normal NaOH. In order to get complete extraction, pre-leaching the soil with sodium acetate was found necessary. The inorganic phosphorus was determined in the NaOH extract by decolorization with bromine water, or by the Kieselguhr method (precipitation of humus with 6N H₂SO₄). Analysis of a large number of widely different soils showed substantial agreement between the two methods of decolorization. On the average, after active calcium was removed, about 60% of the alkali soluble phosphorus was organic. The organic phosphorus in these soils made up about 25% of the total. Dean's study of the solubility of inorganic phosphatic material in sodium hydroxide revealed that tricalcium phosphate and

apatite were relatively insoluble, while iron, aluminum, mono- and di-calcium phosphates were soluble.

Wrenshall and others (8, 9, 32, 33, 34) at Macdonald College in Canada have made some very extensive studies on the various procedures for the extraction and separation of organic phosphorus in soils. They finally concluded that hot 5 percent ammonium hydroxide, preceded by leaching with N HCl, gave the most complete extraction. During the course of their work they showed that phytic acid forms two distinct ferric salts, having formulas corresponding approximately to $(C_6H_{10}(PO_4)_6)_3Fe_3$ and $C_6H_6(PO_4)Fe_4$. They also found a compound of aluminum with phytic acid which was insoluble in an acid solution. Their studies on the decomposition of some of the organic phosphorus compounds in soil cultures tend to indicate that soil organic phosphorus represents an accumulation of stable forms and not a labile fraction maintained by synthetic activities. It was believed to be the inactive end product of soil processes and hence relatively unavailable to both plants and microorganisms. They point out that soil conditions influence the degree of accumulation to a considerable extent. This is demonstrated by the fact that their analyses showed infertile, acid soils to contain a higher percentage of organic phosphorus than the neutral or calcareous soils. It was pointed out that liming might hasten the decomposition of organic phosphates thereby improving the PO_4 status. Phytin being one of the compounds that accumulates in soil was believed to enter into insoluble combinations with sesquioxide constituents under acid condition, thereby becoming resistant to enzymatic hydrolysis.

Pearson and Simonson (20) studied seven typical Iowa soil profiles to determine the amount and distribution of organic phosphorus therein. They found that the amounts varied from 205 p.p.m. to 393 p.p.m. in the

surface layers but they were as low as 8 p.p.m. in the C horizon of some soils. The proportion of total phosphorus present in organic form ranged from 35.4 percent in the plow layer of the Wiesenboden profile to 72.6 percent in the A₁ horizon of the Fayette silt loam. The ratios of organic phosphorus to organic carbon and nitrogen varied considerably within individual profiles, however, smaller variations occurred in the N:P ratio than in the C:P ratio.

Bower (4) separated phytin from the soil in large enough quantities to study its composition. He found 25 to 35 percent of the organic phosphorus in three Iowa soils to be phytin and another 11 to 15 percent to be phytin derivatives. Since he was able to isolate some of the phytin derivatives, he concluded that the organic phosphorus was decomposing but at a rather slow rate. The phytin derivatives precipitated as their calcium salts had an inositol-P ratio corresponding most closely with that of inositol triphosphate.

EXPERIMENTAL PROCEDURE

Methods of Analysis

In 1911, Fraps (10) showed that organic phosphates were soluble in ammonia after extraction with hydrochloric acid. This was probably the first successful separation of organic from inorganic soil phosphorus. Later Potter and Benton (23) originated a method for distinguishing between these two types of phosphorus in soils. Many others (2, 6, 7, 9, 18, 19, 24, 35) have made slight modifications in technique and have improved the original methods used by the earlier investigators.

Essentially the methods used by most investigators consist of digestion of a sample of soil in a standard quantity of ammonia or sodium hydroxide after removal of calcium with either dilute acid or sodium acetate. The soil is filtered out of suspension, and the phosphorus is determined by the Deniges' method. It is determined in one decolorized aliquot portion before ignition, and in another, not decolorized, after ignition. The difference between the quantities found in the two aliquots is considered to be organic phosphorus.

Several methods have been used for decolorizing the soil extracts. Dean (6) used bromine water and the Kieselguhr method (precipitation of humus with $6\text{NH}_2\text{SO}_4$) and found very good agreement between the two methods. He found the bromine water did not oxidize the organic phosphorus strongly enough to break it down. Pearson found carbon black to be more satisfactory for decolorization.

In general, the method suggested by Pearson (19) with some modification was used in this study. Instead of ignition to decompose the organic phosphates, a concentrated perchloric acid (70-72% HClO_4) digestion was found to be more expedient.

PROCEDURE FOR THE QUANTITATIVE
DETERMINATION OF ORGANIC PHOSPHORUS IN SOILS

Reagents

Ammonium Hydroxide for Extraction. Dilute 35 cc. of concentrated ammonium hydroxide to 1 liter and adjust by titration to approximately 0.5 N.

Sulfuric Acid for Adjusting Acidity. Dilute concentrated sulfuric acid to 35 times its volume and adjust to approximately 1.0 N.

Ammonium Molybdate-Sulfuric Acid Solution. Dissolve 25 grams of molybdate in water heated to 60° C. and filter. Dilute 275 cc. of concentrated sulfuric acid to 800 cc. Cool both solutions and add the ammonium molybdate solution to the sulfuric acid slowly and with stirring. Cool to room temperature and dilute with water to 1000 cc.

Stannous Chloride Solution. Dissolve 25 grams of stannous chloride in 100 cc. of warm concentrated hydrochloric acid. Dilute to 1000 cc. and filter if cloudy. Fresh solutions of this reagent should be prepared periodically since it decomposes on standing, forming a white ppt. which interferes with the determination.

Standard Phosphate Solution. Dissolve 0.2195 gram of recrystallized potassium dihydrogen phosphate in water and dilute to 1000 cc. Dilute 50 cc. of this stock solution to 500 cc. This solution contains 5 p.p.m. of phosphorus and is diluted as required for preparation of final standards. Cover the 5 p.p.m. standard with a layer of toluene and store in a dropping funnel.

p-Nitrophenol Indicator. Dissolve 0.5 gram of solid p-nitrophenol in 100 cc. of water and filter if cloudy.

Carbon Black for Decolorization. If the carbon black shows a test for phosphorus, wash it on a Buchner funnel with 1 to 1 hydrochloric acid until no test for phosphorus can be obtained in the filtrate. Wash the acid out with water, dry, and pulverize.

Perchloric Acid. 70-72% HClO_4 .

Procedure

Preliminary Extraction of Bases. Weigh duplicate 2-gram samples (1.0 gram if the soil contains large amounts of organic matter) of 60-mesh soil into a 100 cc. beaker, add 25 cc. of 0.1 N hydrochloric acid to each, and let stand for several minutes. If the soil is calcareous or contains perceptible quantities of undecomposed plant tissue, place the beaker on a steam chest at 90°C . for about 5 minutes. Filter through phosphorus-free paper and wash the soil with small portions of 0.1 N acid until no test for calcium can be detected in the filtrate; this generally requires six to eight washings. Make the acid filtrate to a volume of 200-cc. in a volumetric flask and save.

Extraction of Phosphorus. Transfer the acid-washed soil with the filter paper to a 500-cc. Erlenmeyer flask graduated at 400 cc. Add 200 to 300 cc. of 0.5 N ammonium hydroxide, stopper, and shake vigorously until the filter paper is thoroughly shredded. Rinse the stopper and sides of the flask and make to volume with 0.5 N ammonium hydroxide. Close the flask with a rubber stopper fitted with Bunsen valves and digest in an oven at 89° to 91°C . for 16 to 18 hours. By the use of these valves the loss of ammonia during digestion is reduced to a minimum.

After digestion is completed, cool the flask in a running water bath to room temperature and add 5 grams of ammonium chloride. Adjust

the volume in the Erlenmeyer to the 400-cc. mark, mix thoroughly by shaking, and let stand for a few minutes until the bulk of the suspended material settles out. Decant through a close, phosphorus-free filter paper, discarding the filtrate as long as any suspended material can be detected in it. Pipet aliquot portions of the clear filtrate into two thoroughly weathered 100-cc. beakers and add proportionate amounts of the acid solution from extraction of bases to each. The size of the aliquots taken depends upon the amount of organic phosphorus present in the soil; in most cases 20 cc. of the ammonium hydroxide solution and 10 cc. of the acid extract are satisfactory. Inorganic phosphorus is then determined in one aliquot and total phosphorus in the other.

Determination of Inorganic Phosphorus in Extract. Add 4 cc. of 1.0 N sulfuric acid and a small amount of phosphorus-free carbon black—usually about 0.2 gram, depending upon the amount of organic matter in solution—to one of the aliquots. Swirl the beaker a few times to mix the suspension thoroughly; filter, rinse the beaker, and wash the paper five times with small portions of water. The carbon will creep over and cause difficulty in the phosphorus determination if the funnel is filled more than half full. Add 2 cc. of ammonium molybdate and adjust the volume to exactly 50 cc.

Prepare standard phosphorus solutions by diluting 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 cc. of the 5 p.p.m. solution to 40 cc. Add 4 cc. of 1.0 N sulfuric acid, 0.12 gram of ammonium chloride for each 10 cc. of the ammonium hydroxide extract taken for analysis, and one drop of indicator, and adjust the acidity just to the yellow color of p-nitrophenol with 1-3 ammonium hydroxide. Add 2 cc. of the ammonium molybdate, dilute

to 50 cc., and develop the color by the addition of 3 drops of stannous chloride. Compare the standard solutions by obtaining a light absorption reading with a photelometer. Prepare a curve by plotting the photelometer readings against the concentration of the standard solutions. Develop the color in the soil extracts and compare on the photelometer. If 10 and 20 cc. aliquots are taken from the HCl and NH_4OH extractions, multiply the figures taken from the curve by ten to get parts per million of phosphorus in the soil.

Determination of Total Phosphorus in Extract. Evaporate the second aliquot to dryness on a steam plate. Add 2 cc. of perchloric acid (70-72% HClO_4) and cover with a watch glass. Digest on a gas or electric hot plate for 30 minutes or until the residue is white. The solution should boil slowly. When digestion is complete, remove the beaker from the hot plate, cool, and wash the lower surface of the watch glass into the beaker. Make the solution up to 40 cc. and neutralize to the yellow color of p-nitrophenol with 1-3 NH_4OH . Add 2 cc. of ammonium molybdate and determine phosphorus as before. Organic phosphorus is found by subtracting inorganic phosphorus from total phosphorus in the ammonium hydroxide and acid extracts.

DETERMINATION OF pH

Determination of pH was made using a Leeds and Northrup Glass Electrode.

DETERMINATION OF ORGANIC MATTER

Organic matter was determined by a modification of the Schollenberger procedure (Soil Science, Vol. 31, 483) which is essentially a wet combustion method using a chromic acid-sulfuric solution for oxidation of the soil organic matter. The optimum temperature for oxidation of the soil organic matter without decomposition of the reagent occurs between 160° and 170° C.

DETERMINATION OF TOTAL PHOSPHORUS

The colorimetric procedure developed by Shelton and Harper (25A) was used to determine total phosphorus. Briefly, the soil is digested with perchloric acid, the insoluble residue filtered off, the filtrate made up to volume, and to an aliquot, sodium molybdate is added. The molybdic acid combined with the phosphate ion is reduced with hydrazine sulfate in a slightly acid solution to form a blue color which increases in intensity in proportion to the quantity of phosphorus present.

Source of Material

The soil samples used in this study were obtained from fifteen counties in the State. A virgin and a cultivated sample from each of 30 different soil types was collected, the location being shown in Table 1. The cultivated sample was collected as nearly adjacent to the virgin sample as was possible in an effort to minimize the variance in parent material. All of the soils shown in Table 1 were taken to a depth of six inches.

Three soil types were chosen for a study of the distribution of organic phosphorus in the soil profile. The Oswego silt loam and the Newtonia very fine sandy loam were developed under grass vegetation; the Bowie very fine sandy loam was developed under timber. All three were virgin soils. Their location is shown in Table 4.

Four samples of alfalfa hay were selected from the experimental plots on the Experiment Station Farm at Stillwater, Oklahoma, for a study of the organic phosphorus in forage grown on soil receiving supplemental treatments of lime and phosphate fertilizers.

EXPERIMENTAL RESULTS

Loss of Total Phosphorus, Total Nitrogen,
and Organic Phosphorus as a Result of Cultivation

It is a well-known fact by all students of agriculture that as soon as a soil is plowed and utilized for the production of cultivated crops, the virgin fertility of that soil begins to decrease. The natural protective covering provided by nature has been destroyed, and unless soil improving practices are adopted, and generally they have not been, the productive capacity of the soil will decline. To the casual observer this decline is less pronounced than to the laboratory analyst.

Total phosphorus and total nitrogen have been extensively studied and the rate of loss of these substances has been adequately reported. However, no previous attempt has been made to show the rate of organic phosphorus lost as compared to the rate of total phosphorus and nitrogen lost. Several investigators have reported the quantity of organic phosphorus in soil and its distribution in the profile. Pearson (20) found the organic phosphorus content of seven Iowa soil profiles to be from 27.2 to 65.2 percent of the total phosphorus. In the plow layer this amount was shown to be 205 p.p.m. to 393 p.p.m. In the C horizon only 8 p.p.m. organic phosphorus was found in some of the soils. These figures show that quite a large percent of the total phosphorus in the soil is in an organic form, but no conclusions have been made to show this type of phosphorus to be either more or less available for plant use. The experiments of Whiting and Heck (31) show that such crops as oats and red clover would utilize phytin as a source of phosphate when grown in sand cultures. Pierre and Parker (21) on the other hand found that plants grown in soil-solution cultures would not take up phosphates

of the organic form. If this line of reasoning were followed, organic phosphates would be expected to accumulate in the soil, and the quantity found in the cultivated soil should be comparable to the quantity found in virgin soils.

From the results of the analysis of a heterogeneous sampling of soils in the State (Table 1), it is evident that the organic phosphates are being used either directly or indirectly by growing plants as a source of phosphorus. Plant physiologists generally recognize that plants take up phosphorus chiefly as the phosphate ion. Additional information is needed to determine whether or not the organic phosphates, in order to be utilized, must first be mineralized. The chemical analyses made during the course of this study show the quantity of organic phosphorus in virgin soils to vary from 45 to 173 p.p.m., and from 30 p.p.m. to 150 p.p.m. in cultivated soils. The average for the virgin 114.1 p.p.m., the cultivated 92.1 p.p.m. On a percentage basis, the average content of organic phosphorus in the virgin soils was 45.8 percent of the total P, and 47.0 percent in the cultivated soils. These figures show that the average ratio of organic phosphorus to total phosphorus is remaining fairly constant. This indicates that the organic phosphorus compounds are being removed at a rate comparable to that of the inorganic phosphorus compounds. It is not known whether the organic phosphates are being absorbed directly as an organic phosphate ion or whether they are first mineralized. However, Bower (4) shows that the more complex organic phosphates are being decomposed at a rather slow rate. Whereas he found 25 to 35 percent of the soil organic phosphorus to be phytin, only 11 to 15 percent was separated as phytin derivatives.

Several investigators (4, 8, 15, 28) have pointed out that in some soils organic phosphates are fixed in a very insoluble form. Since

TABLE 1. SOIL TYPE, LOCATION, AND CHEMICAL ANALYSIS OF SAMPLES COLLECTED FOR STUDY FROM FIFTEEN COUNTIES IN OKLAHOMA.

Sample No.	County Location	Soil Type	Virgin or Cultivated	pH	% O.M.	% N	Total P p.p.m.	Organic Phosphorus p.p.m.	Percent Organic Phosphorus
3	Garfield	Grant vfls	V	6.4	2.54	.151	248	91	36.7
4	Garfield	Grant vfls	C	6.0	2.19	.097	177	80	35.2
5	Garfield	Pond Creek sil	V	6.2	1.74	.164	268	91	34.0
6	Garfield	Pond Creek sil	C	5.9	1.69	.101	193	66	34.2
7	Major	Pratt loamy fs	V	7.0	2.89	.082	95	45	47.9
8	Major	Pratt loamy fs	C	6.7	.80	.048	76	30	39.5
9	Major	Foard sil	V	6.7	2.46	.155	284	90	31.7
10	Major	Foard sil	C	6.4	1.54	.115	216	75	34.7
19	Carter	Durant cl	V	6.0	2.42	.120	248	87	35.1
20	Carter	Durant cl	C	6.4	2.08	.118	170	73	42.9
21	Murray	Newtonia vfls	V	6.3	4.29	.166	357	155	43.4
22	Murray	Newtonia vfls	C	6.3	1.61	.090	218	90	41.3
23	Murray	Denton cl, deep phase	V	6.0	5.30	.231	318	120	37.7
24	Murray	Denton cl, deep phase	C	6.0	3.23	.155	246	95	38.6
25	Nowata	Summit sil	V	6.7	----	.204	300	113	37.6
26	Nowata	Summit sil	C	6.0	----	.172	257	93	36.2
29	Kiowa	Tillman cl	V	6.3	2.28	.147	342	173	50.6
30	Kiowa	Tillman cl	C	6.6	1.53	.099	213	150	70.4
33	Greer	Hollister sil	V	7.3	2.42	.136	362	145	40.0
34	Greer	Hollister sil	C	7.2	1.90	.128	300	130	43.3
35	Greer	Tillman sil	V	6.9	2.40	.151	312	150	48.1
36	Greer	Tillman sil	C	6.5	1.87	.094	234	115	49.1
37	Garfield	Oswego sil	V	6.2	2.29	.151	181	115	63.5
38	Garfield	Oswego sil	C	6.1	1.37	.086	135	80	59.3

TABLE 1. (Continued)

Sample No.	County Location	Soil Type	Virgin or Cultivated	pH	% O.M.	% N	Total P p.p.m.	Organic Phosphorus p.p.m.	Percent Organic Phosphorus
39	Garfield	Kirkland sil	V	6.0	2.66	.155	230	125	54.3
40	Garfield	Kirkland sil	C	5.9	2.22	.118	168	95	56.5
41	Garfield	Renfro sil	V	6.1	2.07	.155	177	116	65.5
42	Garfield	Renfro sil	C	5.8	1.15	.088	115	60	52.2
43	Garfield	Vernon vfls	V	6.1	1.79	.115	116	92	79.3
44	Garfield	Vernon vfls	C	6.0	1.26	.065	83	70	84.3
45	Noble	Vanoss sil	V	6.4	3.72	.178	329	140	42.6
46	Noble	Vanoss sil	C	6.2	1.71	.115	212	120	56.6
47	Noble	Summit, cl	V	6.4	4.72	.246	305	100	32.7
48	Noble	Summit cl	C	6.7	3.02	.153	234	70	29.9
49	Noble	Norge sil	V	5.9	4.11	.185	220	125	56.8
50	Noble	Norge sil	C	5.4	1.71	.105	135	87	64.4
51	Noble	Labette, sicl	V	5.9	3.56	.176	183	115	62.8
52	Noble	Labette sicl	C	5.3	1.85	.122	155	105	69.1
53	Noble	Canadian fsl	V	6.6	2.62	.120	250	135	54.0
54	Noble	Canadian fsl	C	6.3	1.69	.082	245	130	53.1
55	Noble	Polo loam	V	6.0	2.98	.189	201	112	55.7
56	Noble	Polo loam	C	6.0	2.02	.126	170	100	58.8
57	Noble	Otoe clay	V	7.4	4.20	.248	311	145	47.0
58	Noble	Otoe clay	C	6.9	3.91	.262	286	145	51.0
59	Noble	Labette sil	V	5.8	3.84	.197	213	88	41.3
60	Noble	Labette sil	C	5.4	2.61	.136	197	80	40.6
61	Kay	Labette sicl	V	5.5	3.20	.197	192	70	36.5
62	Kay	Labette sicl	C	5.2	1.90	.128	150	70	46.7
2341	Beaver	Richfield sil	V	7.4	3.42	.185	490	145	29.6
2343	Beaver	Richfield sil	C	7.5	2.20	.110	415	65	15.7
3991	Tulsa	Parsons vfls	V	5.8	3.40	.120	205	85	41.5
3992	Tulsa	Parsons vfls	C	5.8	2.47	.110	192	85	44.3

TABLE 1. (Continued)

Sample No.	County Location	Soil Type	Virgin or Cultivated	pH	% O.M.	% N	Total P p.p.m.	Organic Phosphorus p.p.m.	Percent Organic Phosphorus
3975	Tulsa	Bates vfsl	V	6.1	4.15	.182	228	140	61.4
3975a	Tulsa	Bates vfsl	C	5.6	2.92	.126	210	135	64.3
4281	Tulsa	Yahola	V	7.6	2.47	.120	380	70	18.4
4284	Tulsa	Yahola	C	7.5	1.24	.060	315	50	16.4
6363	Woods	Miller cl	V	7.8	3.02	.150	535	150	28.0
6364	Woods	Miller cl	C	8.0	2.13	.115	505	145	28.7
6529	Payne	Field "0" (Manured)		5.6	2.32	.119	280	75	26.8
6559	Payne	Field "0"		5.1	1.69	.086	230	95	41.3
8148	Ofuskee	Parsons vfsl	V	6.2	2.60	.107	160	95	59.4
8147	Ofuskee	Parsons vfsl	C	5.2	1.65	.077	140	75	53.6

	<u>Virgin</u>	<u>Cultivated</u>
Average content of Organic Phosphorus in p.p.m.....	114.1	92.1
Average content of Organic Phosphorus in percent.....	45.8	47.0

phytin has been shown to be one of the more abundant of the organic phosphorus compounds in soil, it is probable that it enters into insoluble combination with the sesquioxide constituents in many soils and forms compounds which resist enzymatic hydrolysis and accumulates to a very marked degree. The percentage of organic phosphorus shown in Table 1 indicates that this type of fixation has probably occurred in some virgin and cultivated soils, but usually is more pronounced in the latter.

There has been question as to whether nitrogen and organic phosphorus are chemically combined in certain of the organic compounds in soil. If they are, there is good reason to believe that their decomposition patterns would fall along parallel lines. In an effort to show this relationship the percent loss of each of these constituents from the virgin condition is shown in Table 2. Of the thirty soil types compared, more than two-thirds of them show that the inorganic phosphates are being lost at a more rapid rate than the organic phosphates. This result would be expected since it is known that when inorganic phosphates are available they are taken up readily by plants. However, the rate at which the total phosphorus content of the soil is being lost is only slightly higher on an average basis than the rate at which the organic phosphates are being lost. An average of the thirty soils studied shows total phosphorus lost through cultivation to be 21.61 percent as compared to 19.19 percent of the organic phosphorus lost. The amount of nitrogen lost, however, does not present such a close relationship. The analyses show that in 85% of the soils studied, the percent of nitrogen lost through cultivation was appreciably higher than the percent of organic phosphorus lost. An average for all the soils studied showed that 31.04 percent of the nitrogen has been lost, as compared to

TABLE 2. PERCENT TOTAL PHOSPHORUS, ORGANIC PHOSPHORUS AND NITROGEN LOST THROUGH CULTIVATION.

Sample Number	Soil Type	Percent Total Phosphorus Lost Through Cultivation	Percent Organic Phosphorus Lost Through Cultivation	Percent Nitrogen Lost Through Cultivation
3,4	Grant vfsl	28.6	12.1	35.8
5,6	Pond Creek sil	28.0	27.4	38.4
7,8	Pratt lfs	20.0	33.3	41.5
9,10	Foard sicl	23.9	16.6	25.8
19,20	Durant cl	29.8	16.1	1.7
21,22	Newtonia vfsl	39.4	41.9	45.7
23,24	Denton cl	22.6	20.8	32.9
25,26	Summit sil	14.3	17.6	15.7
29,30	Tillman cl	37.7	13.3	32.7
33,34	Hollister sil	17.1	10.3	5.8
35,36	Tillman sil	25.0	23.3	37.7
37,38	Oswego sil	25.4	30.4	43.0
39,40	Kirkland sil	27.0	24.0	23.8
41,42	Renfro sil	35.0	48.3	43.2
43,44	Vernon vfsl	28.4	23.9	43.5
45,46	Vanoss sil	35.6	14.3	35.3
47,48	Summit cl	23.3	30.0	37.8
49,50	Norge sil	38.6	30.4	43.2
51,52	Labette sicl	15.3	8.7	30.7
53,54	Canadian fsl	2.0	3.7	31.6
55,56	Polo loam	15.4	10.7	33.3
57,58	Otoe clay	8.1	0.0	5.3
59,60	Labette sil	7.5	9.1	30.9
61,62	Labette cl	21.9	0.0	35.0
2341,42	Richfield sil	15.3	55.1	40.5
3991,92	Parsons vfsl	6.3	0.0	8.3
3975,75a	Bates vfsl	7.9	3.6	30.8
4281,84	Yahola	17.1	28.6	50.0
6363,64	Miller cl	20.0	1.0	23.3
8148,47	Parsons vfsl	12.5	21.1	28.0
Averages		21.61	19.19	31.04

the afore mentioned figure of 19.19 percent for organic phosphorus. These figures are significant in that they show the nitrogen compounds in the soil are more rapidly decomposed and more quickly utilized than the phosphorus compounds. They are also significant in that they show that the organic phosphorus reserve in the soil is not being expended as rapidly as the nitrogen reserve. It is unfortunate that more complete information is not known about the soils used in this study, as the history with regard to the time in cultivation, vegetative cover, and type of cropping systems would have added immeasurably to the knowledge of the soil processes that have occurred.

Ratio of Organic Phosphorus to Nitrogen
and Organic Matter in Virgin and Cultivated
Soils

Since it has previously been pointed out in this study that the nitrogen content of the soil is decomposing at a more rapid rate than the organic phosphorus, it naturally follows that the ratio of organic phosphorus to N would narrow as the soils are cultivated. The OP:N and the OP:organic matter ratios are shown in Table 3. The ratio of OP:N varies from 1:8.5 to 1:28.1 in the virgin soils and from 1:6.3 to 1:21.9 in the cultivated soils. The ratio of OP:organic matter varies from 1:132 to 1:457 in the virgin soils and from 1:102 to 1:431 in the cultivated soils.

Other investigators who have studied the OP:N and OP:organic matter ratios in soils have also found a wide variation between soil types. Auten (2) found the OP:N ratios to vary from 1:8 to 1:24, while Pearson (20) found these ratios to vary from 1:7 to 1:11 in the seven soils he studied.

All of the investigation shows that there is less variation in OP:N than there is OP:organic matter.

Distribution of Organic
Phosphorus in Three Virgin Soil Profiles

Soil samples were taken at varying depths to 24 inches from Oswego, Newtonia, and Bowie soil types for a study of the distribution of organic phosphorus in the profile. The analysis of the three soils (Table 4) shows that the percentage of organic phosphorus down through the profile of the Oswego and Newtonia soils is relatively constant to depths of 24 and 18 inches respectively, whereas the percentage of organic phosphorus in the Bowie profile drops from 60 percent in the surface inch to 13 percent in the 10-18 inch layer. This difference in the three soil types may partially be explained by the fact that the Oswego and Newtonia were developed under grass while the Bowie, a coastal plains type, was developed under timber.

The organic phosphorus made up about 45 percent of the total phosphorus in the Oswego profile, 35 percent in the Newtonia, and about 30 percent in the Bowie.

The general trend of the OP:N ratio in the Oswego and Newtonia with increasing depth is similar in that the ratio narrows with depth, however, the converse is true in the Bowie profile where the ratio widens with depth. Figure 2 shows graphically the distribution of organic phosphorus and nitrogen in the profile, where concentration is plotted against depth. It is readily seen from these graphs that the distribution of nitrogen and organic phosphorus in the soil profile follow nearly parallel patterns.

TABLE 3. RATIO OF ORGANIC PHOSPHORUS TO NITROGEN AND ORGANIC MATTER IN VIRGIN AND CULTIVATED SOILS

Sample Number	Soil Type	Organic P : Nitrogen		Organic P : Organic Matter	
		Virgin	Cultivated	Virgin	Cultivated
3,4	Grant vfst	1:16.6	1:12.1	1:279	1:274
5,6	Pond Creek sil	1:18.0	1:15.3	1:191	1:256
7,8	Pratt loamy fs	1:18.2	1:16.0	1:642	1:267
9,10	Foard sil	1:17.2	1:15.3	1:273	1:205
19,20	Durant cl	1:13.7	1:16.1	1:278	1:285
21,22	Newtonia vfst	1:10.1	1:10.0	1:277	1:179
23,24	Denton cl	1:19.3	1:16.3	1:442	1:340
25,26	Summit, sil	1:18.0	1:18.4	---	---
29,30	Tillman, cl	1: 8.5	1: 6.6	1:132	1:102
33,34	Hollister sil	1:10.5	1: 8.8	1:186	1:131
35,36	Tillman, sil	1:10.1	1: 8.2	1:160	1:163
37,38	Oswego sil	1:13.1	1:10.7	1:199	1:171
39,40	Kirkland sil	1:12.4	1:12.4	1:212	1:233
41,42	Renfro, sil	1:13.4	1:14.6	1:178	1:192
43,44	Vernon vfst	1:12.5	1: 9.3	1:194	1:180
45,46	Vanoss sil	1:12.7	1: 9.6	1:265	1:142
47,48	Summit cl	1:24.6	1:21.9	1:472	1:431
49,50	Norge sil	1:14.8	1:12.0	1:329	1:197
51,52	Labette sil	1:15.3	1:11.6	1:310	1:176
53,54	Canadian fsl	1: 8.9	1: 6.3	1:194	1:130
55,56	Polo loam	1:16.9	1:12.6	1:266	1:202
57,58	Otoe clay	1:18.1	1:17.1	1:270	1:290
59,60	Labette sil	1:22.4	1:17.0	1:436	1:326
61,62	Labette, cl	1:28.1	1:18.3	1:457	1:271
2341,42	Richfield sil	1:12.8	1:16.9	1:236	1:338
3991,92	Parsons vfst	1:14.1	1:12.9	1:400	1:290
3975,75A	Bates vfst	1:13.0	1: 9.3	1:297	1:216
4281,84	Yahola	1:17.1	1:12.0	1:353	1:248
6363,64	Miller cl	1:10.0	1: 7.9	1:201	1:146
6529,59	Field "0"	1:15.9*	1: 9.1**	1:309	1:178
8148,47	Parsons vfst	1:10.7	1:10.3	1:260	1:220

* Cultivated soil manured.

** Cultivated soil untreated.

Organic Phosphorus in Alfalfa Hay

Investigation has shown that when green manure crops are plowed under, very little response in the succeeding crop results from the application of phosphate fertilizers. In order to determine the proportion of organic to inorganic phosphates in forage, four samples of alfalfa hay were selected from the experimental plots on the Experiment Station Farm. The samples selected were taken from plots which received no treatment, superphosphate only, superphosphate plus lime, and lime only. Previous studies have shown that the total content of phosphorus in the plant may increase when phosphate fertilizers are applied to the soil. Therefore, it was decided to determine the organic phosphorus content of these samples in an effort to study the effect of fertilizer applications on the synthesis of organic phosphorus compounds in the plant. The analysis data shown in Table 5 indicate that about one-third of the phosphorus in alfalfa plants is in some form of organic combination. The amount ranges from 28 percent in the untreated plots to 36 percent in the plots treated with phosphate only. Lime appeared to have a depressing effect on organic phosphate accumulation in the plant on a percentage basis when applied with superphosphate, but it had an accumulative effect when applied alone.

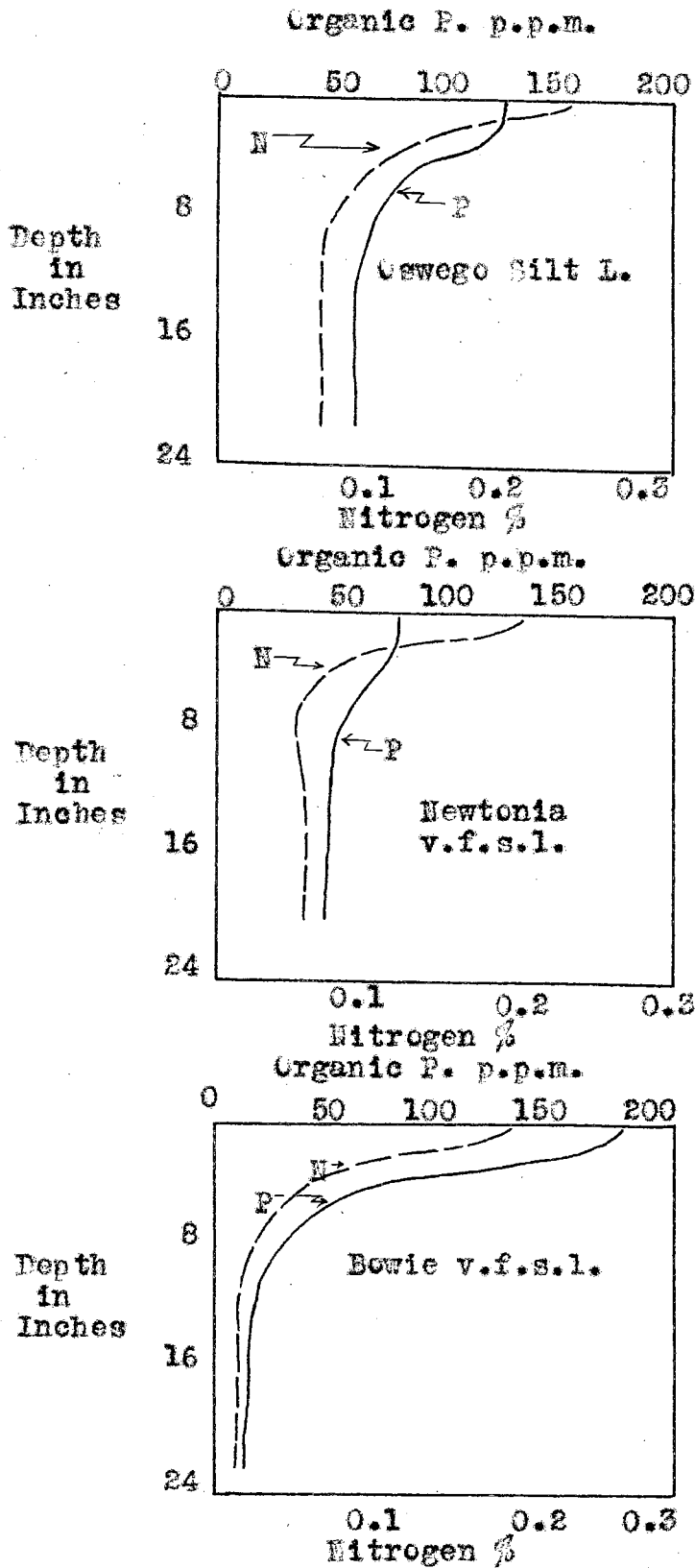
TABLE 4. THE pH, NITROGEN, TOTAL PHOSPHORUS, ORGANIC PHOSPHORUS, PERCENT ORGANIC PHOSPHORUS AND ORGANIC PHOSPHORUS-NITROGEN RATIOS IN THREE SOIL PROFILES.

Soil Type	Depth Inches	pH	% Nitrogen	Total P p.p.m.	Organic P p.p.m.	% Organic P	OP:N
Oswego Silt Loam (Grant County)	0-1	6.7	0.224	255	125	49.0	1:17.9
	1-2	6.5	0.167	230	120	52.2	1:13.9
	2-4	6.0	0.144	200	110	55.0	1:13.1
	4-6	6.0	0.091	180	85	47.2	1:10.7
	6-12	6.2	0.077	145	60	41.4	1:12.8
	12-18	6.6	0.075	150	65	43.3	1:12.1
	18-24	7.2	0.070	165	65	39.4	1:10.8
Newtonia vfls (Choctaw County)	0-1	7.0	0.193	272	85	31.3	1:22.7
	1-3	6.2	0.134	224	85	37.9	1:15.8
	3-8	5.4	0.054	126	60	47.6	1: 9.0
	8-16	6.4	0.070	143	50	34.9	1:14.0
Bowie vfls (Choctaw County)	0-1	5.4	0.176	280	170	60.7	1:10.4
	1-6	5.3	0.051	160	50	31.2	1:10.2
	6-10	5.4	0.020	100	15	15.0	1:13.0
	10-18	5.1	0.020	100	13	13.0	1:15.3

TABLE 5. THE EFFECT OF LIME AND SUPERPHOSPHATE FERTILIZATION ON THE ORGANIC PHOSPHORUS CONTENT OF ALFALFA HAY.

Sample Number	Treatment	Total Phosphorus p.p.m.	Organic Phosphorus p.p.m.	Percent Organic Phosphorus
2525	No treatment	1380	392	28.4
2522	Superphosphate	1730	632	36.5
2593	Superphosphate plus lime	1950	600	30.8
2600	Lime	1440	470	34.8

FIGURE 2. DISTRIBUTION OF ORGANIC PHOSPHORUS AND NITROGEN IN THREE VIRGINIA SOIL PROFILES.



DISCUSSION

In the surface layers of many soils, 15 to 85 percent of the phosphorus has been found to be organic. Many of the soils in which the concentration of organic phosphorus is high are very deficient in available inorganic phosphorus as determined by extraction with dilute acids.

When plant residues are incorporated in soil a rapid decomposition ensues, through the action of fungi and bacteria. After the utilization of most of the easily decomposed energy material, such as hemicelluloses and cellulose, bacterial decomposition becomes dominant. Thus organic phosphorus in the soil may originate from the organic residues added to soil, and also through synthesis by microorganisms.

The stability of the organic phosphorus like that of the organic matter should probably be regarded as relative rather than absolute, and should gradually become available through decomposition. The data presented in this study indicate that soil conditions influence the degree of accumulation to a considerable extent. Acid soils usually contain a higher proportion of organic phosphorus than the neutral or calcareous soils. The type of vegetation under which soil is developed would no doubt influence the accumulation. More extensive study is needed to make more definite conclusions on this line of reasoning.

Analyses of virgin and cultivated soils under a wide range of conditions indicate that the organic phosphorus compounds are being decomposed at a rate about equal to the rate that the total phosphorus is being lost from the soil.

There is considerable opinion expressed in the literature that the organic phosphates such as phytin are accumulating in the soil

through the interaction with the sesquioxide constituents. Such compounds would no doubt be quite resistant to enzymatic hydrolysis. The partial dephosphorylation of phytin produced phosphoric esters of inositol as Bower (4) concluded they did. There is no evidence regarding the relative stability of these compounds in the soil, but it is possible that they account for a significant part of the organic phosphorus.

Suffice it to say that no one has yet identified nearly all of the organic phosphorus compounds in soil. The possibility exists, therefore, that other phosphorus compounds, yet unidentified, do exist in soils and are made available for plant use as the loss of organic phosphorus in cultivated soils indicates that they are.

SUMMARY

Virgin and cultivated samples of thirty soil types occurring in Oklahoma were analyzed for organic phosphorus. The chemical analysis data presented show the organic phosphorus content to vary from 30 to 173 p.p.m. in the surface six inch depth of soil. The virgin soils averaged 114 p.p.m. as compared to 92 p.p.m. in the cultivated soils. The organic phosphorus averaged 46 percent of all the phosphorus in the soil, with the average of the virgin soils showing slightly less and the average of the cultivated soils slightly more.

In the profile studies the amount of organic phosphorus was fairly constant on a percentage basis in the Oswego and Newtonia types. The OP in these soils averaged 45 and 35 percent of the total phosphorus from the surface to a 24-inch depth. In the Bowie soil the amount of OP as compared to total P dropped from 60 percent in the surface inch to 13 percent in the 10 to 18 inch layer.

The ratio of organic phosphorus in most soils to nitrogen varied from 1:10 to 1:18 with the extremes 1:6 and 1:28. The ratio of OP to organic matter was less regular than the OP:N ratio; it varied from 1:102 to 1:457 with one extreme condition in which the ratio was 1:642.

Four samples of alfalfa hay from experimental fertilizer plots were analyzed for OP by the same method as that used for soils. The amounts varied with treatment. The sample grown with no fertilizer treatment contained 392 p.p.m. or 28.4 percent of the total phosphorus in the plant. Superphosphate, superphosphate plus lime, and lime alone yielded plants containing 632 p.p.m., 600 p.p.m., and 470 p.p.m., respectively, which are 36.5, 30.8, and 34.8 percent of the total phosphorus.

A modified method for the determination of organic phosphorus in soils is described which gives results with a high degree of accuracy.

BIBLIOGRAPHY

1. Aso, K. On organic compounds of phosphorus in soils. *Bul. Col. Agr. Tokio Imp. Univ. (Japan)*, 6(1904) 277-284.
2. Auten, J. T. The organic phosphorus content of some Iowa soils. *Soil Sci.* 13(1921) 117-124.
3. Auten, J. T. Organic phosphorus of soils. *Soil Sci.* 16(1923) 281-294.
4. Bower, C. A. Separation and identification of phytin and its derivatives from soils. *Soil Sci.* 59(1945) 277-286.
5. Conant, J. B. The Chemistry of Organic Compounds, p. 485.
6. Dean, L. A. An attempted fractionation of the soil phosphorus. *Jour. Agr. Sci.* 28(1938) 234-244.
7. Dickman, S. R., and DeTurk, E. E. A method for the determination of the organic phosphorus of soils. *Soil Sci.* 45(1938) 29-39.
8. Dyer, W. J., Wrenshall, C. L., and Smith, G. R. The isolation of phytin from soil. *Science* 91(1940) 319-320.
9. Dyer, W. J., and Wrenshall, C. L. Organic phosphorus in soils : I. The extraction and separation of organic phosphorus compounds from soil. *Soil Sci.* 51(1941) 159-170.
10. Fraps, G. S. Organic phosphoric acid of soils. *Tex. Agr. Exp. Sta. Bul.* 136 (1911).
11. Heck, A. F., and Whiting, A. L. The assimilation of phosphorus from phytin by red clover. *Soil Sci.* 24 (1927) 17-29.
12. Hilbert, G. E., et al. Organic phosphates : I. Fixation studies with three different soil types. *Soil Sci.* 46 (1938) 409-418.
13. Hopkins, C. G., and Pettit, J. H. The fertility in Illinois soils. *Ill. Agr. Exp. Sta. Bul.* 123 (1908).
14. Kelly, W. P. The biochemical decomposition of nitrogenous substances in soils. *Hawaii Agr. Exp. Sta. Bul.* 39 (1915).
15. McGeorge, W. T., and Breazeale, J. T. Studies on iron, aluminum, and organic phosphates. *Ariz. Agr. Exp. Sta. Tech. Bul.* 40 (1932).
16. Mulder. *Über die Bestandtheile der Ackererde.* *Jour. Prakt. Chem.*, Bd. 32(1844) 326.
17. Neubauer, E. Versuche nach der Keimpflanzenmethode über den Unterscheid der Aufnehmbarkeit anorganischer und organischer Phosphorformen. *Landw. Vers. Sta.* 114(1933) 225-294.

18. Odynsky, W. Solubility and distribution of phosphorus in Alberta soils. *Sci. Agr.* 16(1936) 652-664.
19. Pearson, R. W. A procedure for the determination of organic phosphorus in soils. *Ind. and Eng. Chem., Anal. Ed.*, 12(1940) 198-200.
20. Pearson, R. W., and Simonson, R. W. Organic phosphorus in seven Iowa soil profiles: distribution and amounts as compared to organic carbon and nitrogen. *Soil Sci. Soc. Amer. Proc.* 4(1940) 162-167.
21. Pierre, W. A., and Parker, F. W. Soil phosphorus studies: II. The concentration of inorganic and organic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants. *Soil Sci.* 24(1927) 119-128.
22. Plimmer, R. H. A. The metabolism of organic phosphorus compounds. Their hydrolysis by the action of enzymes. *Biochem. Jour.* 7(1913) 43-71.
23. Potter, R. S., and Benton, T. H. The organic phosphorus of soil. *Soil Sci.* 2(1916) 291-298.
24. Schollenberger, C. J. Organic phosphorus of soil. Experimental work on methods for extraction and determination. *Soil Sci.* 6(1918) 365-395.
25. Schollenberger, C. J. Organic phosphorus content of Ohio Soils. 10(1920) 127-141.
- 25A. Shelton, W. R., and Harper, H. J. A rapid method for the determination of total phosphorus in soil and plant material. *Iowa State College Journal of Science*, 15(1941) 403-413.
26. Shorey, E. Nucleic acid in soils. *Science*, n. s. 35 (1911) 390.
27. Shorey, E. G. Some organic soil constituents. U.S.D.A. Bureau Soils Bul. 88 (1913).
28. Spencer, V. E., and Stewart, R. Phosphate studies: I. Soil penetration of some organic and inorganic phosphates. *Soil Sci.* 38 (1934) 65-79.
29. Stewart, Robert. Quantitative relationship of carbon, phosphorus, and nitrogen in soils. *Ill. Agr. Exp. Sta. Bul.* 145 (1910).
30. Truog, E., and Meyers, A. H. Improvements in the Deniges' colorimetric method for phosphorus and arsenic. *Ind. and Eng. Chem., Analyt. Ed.* 1(1929) 136-139.
31. Whiting, A. L., and Heck, A. F. The assimilation of phosphorus from phytin by oats. *Soil Sci.* 22(1926) 477.
32. Wrenshall, C. L., and McKibben, R. R. Pasture studies: XIII. The nature of the organic phosphorus in soils. *Can. Jour. Res.* 15B (1938) 475-479.

33. Wrenshall, C. L., and Dyer, W. J. A method for the determination of organic phosphorus in soils and soil extracts. *Can. Jour. Res. (B)* 17(1939) 199-205.
34. Wrenshall, C. L., and Dyer, W. J. Organic phosphorus in soils: II. The nature of organic phosphorus compounds. A. Nucleic acid derivatives. B. Phytin. *Soil Sci.* 51(1941) 235-248.
35. Yoshida, R. K. Studies on organic phosphorus compounds in soil; isolation of inositol. *Soil Sci.* 50(1940) 81-89.

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