

PHOSPHORUS FRACTIONS IN SOME ARIZONA SOILS

by

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ABSTRACT

Ten soils were selected for testing on the basis of their representation of several important agricultural areas of Arizona and because of the information which was available on these soils.

The phosphorus in the soils was characterized by extracting the soils by two methods: the Chang and Jackson procedure and Metha's procedure.

Statistical analyses were made on the data for the various fractions alone and in combinations with each other versus the yield in grams of dry weight of tomato plants grown in the greenhouse. The only significant correlation found between any variable and yield was that of the ammonium chloride extraction. The amount of phosphorus in that fraction expressed as ppm. phosphorus showed a significant curvilinear correlation with yield of tomatoes. A significant linear correlation also was found with the same variables if soil S-100 was omitted from the analysis. It appears that this soil had some dissimilar physical or chemical properties that influenced the results.

Plant utilization of the discrete soil forms of phosphorus did not appear to be proportionate for all

soils. The ammonium chloride fraction represents the easily soluble portion of several discrete forms of soil phosphorus.

INTRODUCTION

The methods which have been developed for the evaluation of the phosphate fertility status fall into two distinct categories: biological and chemical. The biological methods which are of necessity time consuming have more or less been relegated to the background. For the most part, the majority of the research is concerned with the chemical methods of evaluating the soil phosphorus available to plants.

The chemical methods have primarily followed two approaches. The first method extracts from the soil what is called available phosphorus, i.e., available to plants. The second method attempts to extract discrete forms of the soil phosphates. Procedures adhering to this practice are the so-called fractionation methods.

The methods which employ solutions that extract known forms of phosphates from the soil were developed primarily to aid in the characterization of the soil phosphates. However, recently it has been found that some of the forms which are removed by these methods are correlated with growth of crops and response to phosphorus fertilizers. The phosphorus extracted by these methods in their various forms when evaluated with respect to the amounts present, solubilities of the various forms, and conditions of the

soil may be of benefit in the development of an appropriate soil testing procedure for calcareous soils.

Some of the methods which extract available or easily soluble phosphorus have had moderate success on the calcareous soils of Arizona with respect to crop response. The carbon dioxide-saturated water method and Bray's weak acid-ammonium fluoride method have shown some promise. However, because they still leave something to be desired as a diagnostic technique for evaluating crop response to phosphorus it was felt that a study of the forms of phosphorus present in certain calcareous soils may be helpful in developing the basic knowledge essential for the development of a procedure that can evaluate more adequately soil phosphorus available to plants.

The purpose of this investigation was to fractionate the phosphorus of some calcareous soils of Arizona by using a slight modification of the method proposed by Chang and Jackson. Further, it was hoped that a relationship between growth as exhibited by tomato plants in the greenhouse and the amount of phosphorus present in any one fraction or combination of fractions could be found.

LITERATURE REVIEW

Since an abundance of literature is available on the subject of phosphorus and its relationship to plant nutrition and soil fertility no attempt was made to include references pertaining to all the literature available, but, rather, to select only those articles which were considered of prime importance for development of suitable background information for this thesis problem.

Forms in Which Phosphorus Exists in the Soil

Phosphorus may be present in soils in general in two very broad categories, inorganic and organic. In each of these categories many forms are known to exist. In the alkaline-calcareous soils in Arizona the preponderance of the soil phosphates are found in the inorganic forms, Fuller and McGeorge (19). This does not in any way indicate that the organic fraction is not important as a source of P for plants.

The principal forms in which organic phosphorus exist in the soil are: phospholipids, nucleic acids, and phytin or inositol hexaphosphoric acid. Many seem to regard the organic phosphorus as a characteristic fraction of organic matter; however, Barrow (1) believes that one

should regard organic phosphorus as a varying portion of the organic matter. Barrow stated that alkaline soils always contained less phosphorus in the organic matter than the acid soils tested. This may be true; but, simply because the organic phosphorus was lower does not indicate the quantity or importance of that present. Even if an alkaline soil contains less phosphorus in the organic form it still may contain enough organic phosphorus to be of consequence, in so far as plant response or availability to the plant is concerned. An inspection of Barrow's data showed that indeed this was the case, that is, although there was less organic phosphorus in the alkaline soils in each instance there was sufficient organic phosphorus to be of value as a source of phosphorus for plants.

Fuller and McGeorge (19) stated that the presence of organic phosphorus in the soil solution cannot be ignored in the calcareous soils from Arizona which they tested. They found that an unexpectedly high proportion of the total extractable phosphorus appeared to be organic. For 19 soils they reported 20 to 50 percent of the total phosphorus as organic. An appreciable portion of the water soluble phosphorus on calcareous soils also was found to be organic. In testing the solubility of organic phosphorus in various water to soil ratios they found that as the ratio increased the solubility of the organic phosphorus increased. The highest ratio used was a 20 to 1 dilution.

Sen Gupta and Cornfield (37) studied the effect of the calcium carbonate content in soils on the amount of organic phosphorus that is present. They found that in the 33 calcareous soils tested organic phosphorus varied from 4 to 30 percent of the total phosphorus. A statistical analysis was made of the data and no significant correlation was found between the calcium carbonate content of the soils and the percentage of organic phosphorus.

Since organic phosphorus can account for from 4 to 50 percent of the total phosphorus it is reasonable to believe that the largest part of the phosphorus in calcareous soils is contained in the inorganic form.

The inorganic fraction of phosphorus in soils is very complex and it is of general knowledge that many compounds make up this fraction. In general, though, the inorganic portion of the soil phosphates exist as compounds of iron, aluminum, calcium, and in several mineral forms and as phosphates in the crystal lattice of clays.

Chang and Jackson (7) found that the majority of the phosphorus in calcareous soils is present in the calcium phosphate fraction with very little in the aluminum and iron fraction. They also stated that on addition of phosphate fertilizer the phosphorus is more likely to be combined with calcium and aluminum. They attribute this to the greater activities of calcium and aluminum

ions in the soil. However, they feel that since the iron phosphate is less soluble this is the form which will tend to accumulate.

Chai Moo Cho and Caldwell (6) in their extraction of several soils found that in acid soils the predominant forms of phosphorus were iron and aluminum phosphates. But, on the alkaline soils they found the calcium phosphate form is predominant.

In an attempt to more carefully characterize the form of soil phosphates present in calcareous soils, Sen Gupta and Cornfield (36) used a modification of the method proposed by Chang and Jackson (8). The modification consisted of using a solution of 0.5N acetic acid before the sulfuric acid extraction as proposed by Chang and Jackson. The acetic acid extraction contained the non-apatitic calcium phosphates, whereas the sulfuric acid extraction of Chang and Jackson dissolved all the calcium phosphates.

Sen Gupta and Cornfield studied six soils with varying calcium carbonate content and found that the inert form or forms which is insoluble in any of the extractants contained the most phosphorus. The phosphorus content of the other forms decreased in the following order: the apatites, non-apatites (calcium phosphates), aluminum phosphate, iron phosphate, and the easily replaceable phosphates. In comparison with acid soils both the iron

and aluminum phosphates were less. Their investigation showed that the calcium carbonate content of the soil had no significant effect on the amount of phosphate present as calcium and iron phosphate. However, for the aluminum phosphate fraction there was a highly significant negative correlation. This indicates that as the calcium carbonate content of the soil increases the aluminum phosphate content decreases.

Another form which is known to exist in acid soils to a considerable degree is the exchangeable or adsorbed phosphates. Dean and Rubins (11) found that soils which do not have excessive amounts of calcium or magnesium in the system take up or fix large amounts of phosphorus by substitution of phosphate ions for hydroxyl ions. They also found that the anion exchange capacity had a very definite correlation to the amount of clay present in the soil or the specific surface. The higher the clay content the higher the anion exchange capacity. On the acid soils used the amount of phosphorus adsorbed varied from 2.2 mmols. to 4.1 mmols. per 100 gms. of soil. For alkaline-calcareous soils it is suspected that adsorption of phosphate does not exist in the amounts that occur in acid soils. Greater adsorption in acid soils would be expected because of surface adsorption on the iron and aluminum compounds. In calcareous soils the mass action of the calcium ions would cause the precipitation of the phosphates

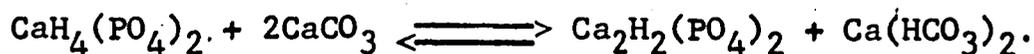
before adsorption could occur. In addition, at higher pH values the hydroxyl ions could compete with phosphorus for the adsorption sites. Nevertheless, to assume that adsorption does not exist at all, in the opinion of the author, would be erroneous. However, the extent to which it does occur is not accurately known.

As has been indicated many forms of phosphates exist in the soil. In order for the knowledge of the existence of these forms to be of any benefit in predicting what might occur in the way of crop response, information about their properties is needed.

Availability of Forms Present

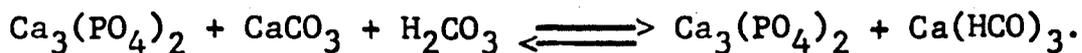
Since it has already been pointed out that the alkaline-calcareous soils contain large amounts of calcium phosphate it may be well to investigate the effect, if any, that calcium carbonate has on the availability of this form of phosphorus.

In testing the availability of phosphates in calcareous soils Breazeale and Burgess (5) found that mono-calcium phosphate added to these soils quickly reverted to the most insoluble form possible under these conditions. The reaction may be written as follows:



They found that this reaction preceded quickly to completion from left to right and is only slightly reversible. Furthermore, they found that the dicalcium phosphate form is stable in the presence of an excess of calcium carbonate and does not go into the tricalcium phosphate form.

In order to further characterize the reactions of the calcium phosphates, Breazeale and Burgess studied the effect of calcium carbonate addition to an aqueous solution of finely ground rock phosphate (assumed to be primarily tricalcium phosphate). They found that calcium carbonate has little effect on the solubility of the phosphate and that upon the addition of carbon dioxide into the suspension the reaction was as follows:



This indicates that the tricalcium phosphate does not enter appreciably into this reaction. Hence, practically no phosphorus is brought into solution as long as calcium carbonate is present. The solubility of calcium bicarbonate is over 1000 ppm. in pure water while that of tricalcium phosphate is very low, approximately 20 ppm. Thus, in a solution containing both, one would expect less phosphorus in solution than with tricalcium phosphate alone.

When studying the phosphate solubility in some unproductive calcareous soils McGeorge and Breazeale (28) showed close agreement between the solubility of phosphate in distilled water and fertility as well as with performance of these same soils in pot experiments. They felt that the velocity of solution was of greater importance than the removal of some of the phosphates with an extraction. They also found that the presence of soluble salts in the soil solution depressed the solubility of the phosphate.

In a later study on the solubilities of iron and aluminum phosphate, McGeorge and Breazeale (29) found that in carbon dioxide-saturated water vivanite, lazulite, wavellite and dufrenite were soluble at rates of less than 0.3 ppm. They also studied the solubility of phytin phosphorus. A 0.25 gram sample of phytin was placed into 500 ml. of CO₂ free water and 9 ppm. phosphorus was found in the extract. The presence of calcium carbonate had no effect on the solubility of the phytin phosphorus, and as the pH value increased the solubility increased.

Laverty and McLean (27) state that in studying the availability of the forms present in the soil some degree of error might arise because of the differences in solubilities of freshly formed and more aged crystals of the phosphate compounds. This would account for some

differences observed in the results of many investigators when they attempt to correlate the easily soluble phosphorus with plant growth.

Studies Made with Solutions Which
Extract "Available Phosphorus"

One of the first investigators to attempt a determination of what is called "available phosphorus" was Truog (41). He proposed the use of an extracting solution of 0.002 N H_2SO_4 buffered with $(NH_4)_2SO_4$ to a pH value of 3.0. The solvent to soil ratio was 200 to 1 and the period of extraction was 30 minutes. This solvent he felt removed the readily available phosphorus by simulating the approximate strength of the solvent operating in plant feeding.

Morgan (32) developed what he termed a "universal soil extraction solution" which consisted of acetic acid buffered with sodium acetate. This method was not limited to phosphorus alone, but by using this extracting solution one could determine NO_3 , NH_4 , K, Ca, Mg, Al, Mn, and Fe, to name a few. He states that the test for phosphorus indicates the level of more readily available phosphorus in the soil whether it is native or a residue from previous applications of phosphorus. It is implied from the report that the test was designed for acid soils.

In testing the solubility of phosphorus in chemical extractants as indices to available phosphorus Thompson

and Pratt (40) tried to ascertain if some chemical extracting procedure could be used to determine the phosphorus available to plants in greenhouse cropping. Among extracts used were Bray and Kurtz's extracting solutions-- numbers 1 and 2-- , Olsen's sodium bicarbonate method, 1 N sodium hydroxide, 1 percent citric acid solution, 1 percent ammonia (buffered at pH value 5.1), and Truog's 0.002 N sulfuric acid solution. They tested both acid and alkaline soils and found that no one method could be related to plant growth over all types of soils. They suggested that no method be used until the method has been calibrated with plant and soil data from the area where it is to be used. Where uniformity of parent material occurs, several of the solutions might be accurate for measurements of available phosphorus. However, for heterogeneous areas many of the same solutions can not be used successfully.

As has been pointed out the determination of the available phosphorus in any one group of soils by different methods frequently result in conclusions that differ with respect to the phosphorus status of the soils concerned. Chang and Juo (9) have attempted to characterize available phosphorus in relation to the forms of phosphates in the soil. It is known that available soil phosphorus determined by a chemical method includes all forms of

inorganic and organic phosphorus although calcium, aluminum and iron phosphates predominate. They concluded the total and relative amount of phosphorus dissolved from the various forms of phosphates in soils depend on the relative amounts of these forms present and the relative solubilities of the phosphates in the extracting solution. Therefore, when two extractants similar in selectivity of dissolution are applied to a group of soils with similar phosphate distribution it is most likely that the values of available phosphorus determined by those two methods will be highly correlated.

When Chang and Juo tested seven different extracting solutions, they found that for soils dominant in calcium phosphates the following were highly correlated with each other. They were Olsen bicarbonate and Bray no. 1, Bray no. 2 and no. 4, Bray no. 2 and North Carolina, Bray no. 2 and Truog, and Bray no. 4 and North Carolina. Peech's acetic acid was not correlated with any of the other methods in the study. Thus it was concluded that the methods which were correlated with each other extract similar forms of phosphates. The methods that were correlated did not necessarily extract the same amounts of phosphorus.

Chemical solvents can distinguish between phosphates which have a high availability, but they do not measure the availability of the phosphorus in the minerals.

According to Fraps and Fudge (18), the most a chemical method can do is ascertain the quantity of phosphorus dissolved by the solvent used. "From the chemical data and other data from appropriate and sufficient experimentation certain conclusions can be drawn as to the ability of the soils to supply phosphorus to plants, but any claim that a chemical method measures the availability of the phosphates in the soil is not supported by the facts."

Attempts have also been made to obtain a correlation between crop response and various phosphorus compounds in the soil by use of selective extraction solutions. Such methods appear to be a less empirical approach to the problem since a knowledge of the nature of the various phosphate compounds in a soil, together with a knowledge of the conditions under which these different forms are available to the growing crop, gives a much more general means of assessing availability (44).

The Use of Selective Extractants

Fisher and Thomas (17) were among the early investigators of selective extractants for the determination of phosphates in the soil. They used two acid solutions with selective extraction properties buffered to maintain a constant pH value in either acid or alkaline soils.

Solubility studies showed a similarity between amounts of phosphorus obtained from soils and the amounts obtained from pure phosphate materials.

To aid in the study of the distribution of the forms of soil phosphorus Dean (12) proposed an extraction procedure which consisted of three extracts and a separation into four fractions. The extracts consisted of a sodium hydroxide soluble inorganic and organic portion, sulfuric acid soluble fraction, and an insoluble fraction of unknown composition. He found that phosphorus added to calcareous soils tended to accumulate in the acid soluble but alkali insoluble forms which he designated as apatites and calcium phosphates.

Ghani (20) proposed a fractionation procedure which divided the soil phosphates into five separate fractions. They were an acetic acid soluble, an alkali soluble inorganic, an alkali soluble organic, an acid soluble, and finally a fraction which is insoluble in any of these extracting solutions. The fractions consisted of calcium phosphates, iron and aluminum phosphates, organic phosphorus, apatites, and phosphates which he felt were an integral part of the clay, respectively. This procedure closely parallels the one proposed by Dean(12).

In subsequent studies Ghani (21) found that by using a compound (8-hydroxyquinoline) in the acetic acid extractant he could effectively block the refixation of phosphorus from a mixture of basic iron and mineral phosphates. With increasing pH-value the blocking effect becomes less and less until in neutral or alkaline soils there was no effect.

In an attempt to characterize the soil phosphates with only two extractants Williams (44) used an acetic acid extractant containing 1 percent 8-hydroxyquinoline and a 1 N sodium hydroxide solution. The first extraction was believed to contain water soluble phosphate, mono-, di-, and tricalcium phosphates, hydroxy and carbonate apatites, magnesium and manganese phosphates and to some extent chloro and fluoro apatites. The second extraction contained adsorbed, iron, and aluminum phosphates as well as organic phosphates. The remainder was insoluble in either extract and consisted of chloro and fluoro apatites and phosphates included in the crystal lattice. He found that the recovery of phosphate added to the soil was almost complete at all pH values. In acid soils phosphate tended to be fixed in the iron and aluminum fraction and in alkaline soils the most of the fixation was due to precipitation with calcium.

It has been pointed out in previous discussion that the conventional methods for determination of available phosphorus appear to extract a portion of all chemical forms having either high solubility or high specific surfaces. In contrast, the fractionation procedures attempt to separate the soil phosphates into the total amounts of each of the discrete chemical forms present. Chai Moo Cho and Caldwell (6) have pointed out that the fractionation of soil phosphates into discrete forms is almost impossible; however, they felt that the fractionation of soil phosphates into general compounds is possible and would be helpful in understanding the behavior of soil phosphates. One method which has received rather widespread use is the fractionation procedure as proposed by Chang and Jackson (8).

Chang and Jackson classified the inorganic soil phosphates into four main groups. The groups were the calcium phosphates, aluminum phosphate, iron phosphate and the reductant soluble phosphates. They preceded the extraction procedure with an ammonium chloride wash which was to contain any easily soluble phosphorus if it existed in the soil.

They employed their method of extracting the soil phosphates in the study of the soil phosphate fractions of some representative soils. On the calcareous soils

tested they found that the majority of the phosphorus was present in the calcium phosphate fraction with very little in the iron and aluminum fractions. They also reported that only trace amounts of phosphate could be found in the reductant soluble iron phosphate and occluded aluminum phosphate fractions.

El Bagouri (15) used the Chang and Jackson procedure to fractionate some calcareous soils in a study of the effect of soil carbonates on the availability of native and added phosphorus. He found only trace amounts of phosphorus in the sodium hydroxide extract, which removes the iron phosphate. A high significant correlation was found between phosphorus uptake by oats and the amount of phosphorus present in the ammonium fluoride extract, which was a combination of the aluminum phosphate and the phosphates soluble in the ammonium chloride wash solution.

Laverty and McLean (27) used the Chang and Jackson procedure in testing the factors affecting yields and uptake of phosphorus by different crops. They felt that the results from the fractionating method could be an useful tool in studying the fate of phosphate applied to soils.

Studies on the Effectiveness of the
Extractants Used in the Chang
Jackson Procedure

Previous and subsequent to the method proposed by Chang and Jackson much work has been done with the selective extractants used in this method on an individual basis. In a study of the role of fluoride ion in the release of phosphate adsorbed by aluminum and iron hydroxides Turner and Rice (42) found that neutral NH_4F reacted with $\text{Al}(\text{OH})_3$ gels to form $(\text{NH}_4)_3\text{AlF}_6$ and the phosphate adsorbed by these gels was more or less completely released by the action of the fluoride. On the other hand, $\text{Fe}(\text{OH})_3$ gels were apparently not attacked by the fluoride and the phosphate adsorbed was not appreciably released.

A modification of the procedure by Chang and Jackson was proposed by Glenn et al. (22). They suggested that the aluminum phosphate be extracted with 0.5 N NH_4F at pH value 8.0 to 8.5 instead of pH value 7.0. This modification was found to extract a minimum of the iron phosphate. The iron phosphate was subsequently extracted with sodium hydroxide in the normal manner, except the time of extraction was shortened to 9 hours instead of the originally proposed 17 hours. The removal of the iron phosphate was found to be complete after the 9 hour period. The reductant soluble iron and aluminum phosphates were

extracted after the alkali extraction and this was followed by the sulfuric acid extraction. They found that the dithionate-citrate and the ammonium fluoride extractants solubilized only negligible amounts of the calcium phosphates from natural soils containing much calcium phosphate.

In another study Hsu et al. (24) showed that on the basis of the solubility products the solubility of aluminum phosphate in the Al-P, $\text{Al}(\text{OH})_3$, $0.5 \text{ N NH}_4\text{F}$ system is at a minimum at pH value 8.0, but even at this pH value the solubility is high enough (0.1 moles per liter) to insure complete extraction of aluminum phosphate from soils. Similar calculations for iron phosphate in an analogous system shows a minimum solubility at pH value of 7.4 (less than 10^{-4} moles per liter). However, the experimentally determined pH value of minimum solubility of iron phosphate in this system was pH value 8.5. Experiments with laboratory preparations of phosphates and hydroxides of aluminum and iron showed complete dissolution of aluminum phosphate by $0.5 \text{ N NH}_4\text{F}$ in one hour over a wide pH range. The amount of iron phosphate dissolved increased with time of extraction exceeding the one hour necessary to dissolve all of the aluminum phosphate.

MATERIALS AND METHODS

Selection of Soils for Analysis

Since the purpose of this investigation was to characterize the phosphorus content of some calcaerous soils of Arizona, soils were selected for which the greatest amount of information was available.

In evaluating three chemical methods for determining the "available phosphorus" in Arizona soils Jones (26) tested soils from different agriculturally important areas in Arizona. The data which he obtained for these soils consisted of the paste pH value, the calcium carbonate content of the soils, the yield for tomatoes (dry weight), and the total uptake of phosphorus in the tissue of the tomatoes grown in pot culture on these soils. These soils were available and therefore it was possible to use them for subsequent analyses. See tables (1) and (2).

Another soil for which similar results existed was also selected for testing. This soil had been used in a greenhouse study at the University of Arizona. The yield in grams of dry weight of plant material is for

TABLE 1.--Analyses of Soils Used in Greenhouse Study.*

Soil	Bray's P-1 Extractable P in ppm	Paste pH Value	Percent Calcium Carbonate
S-10	44.2	7.69	.63
S-20	47.2	7.91	11.10
S-30	41.2	7.71	1.25
S-40	26.5	7.67	4.36
S-50	41.0	7.89	2.27
S-60	21.0	7.74	2.42
S-80	33.5	7.79	4.27
S-90	6.0	8.33	4.68
S-100	53.0	7.77	8.21
S-110	52.0	8.29	7.95

*Data taken from Jones (26).

Note: Data expressed in air dry soil.

TABLE 2.--Yield of Tomatoes Grown in Pots in the Greenhouse.

Soil	Sample Number			Mean
	1	2	3	
	g./pot			
S-10	4.10	4.75	4.98	4.01
S-20	3.46	3.71	4.00	3.72
S-30	5.63	4.65	4.53	4.94
S-40	1.51	1.63	1.22	1.45
S-50	4.11	3.96	4.50	4.19
S-60	2.38	2.14	2.33	2.30
S-80	2.10	2.11	2.39	2.20
S-90	0.16	0.11	0.12	0.13
S-100	4.22	3.64	3.69	3.85
S-200	3.50	3.23	3.18	3.28

Data taken from Jones (26) and Tucker, Professor of Agricultural Chemistry and Soils, University of Arizona (unpublished data).

tomatoes grown in pot cultures in the greenhouse. In all cases the yields used are means of three replications.

The soils which were selected are representative of several rather important agricultural areas and do not necessarily represent different soil classifications. See table (3).

Determination of Total and Organic Phosphorus

The method used to determine both the organic and total phosphorus content of the soil was proposed by Metha et al. (31). They tested the accuracy of the method against three other methods and concluded that their method was more accurate for determining organic phosphorus.

Their conclusion was based on the premise that if more organic phosphorus is found then the method is more accurate. In each of their comparisons their method extracted more organic phosphorus.

The phosphorus was determined in each of the fractions colorimetrically by the method proposed by Dickman and Bray (14) using Bray's P-c solution as a reducing agent for the development of the color. The organic phosphorus content of the soils is then ascertained by

TABLE 3.--Identification of Soils

Soil No.	Classification	Location
S-10*	Adelanto sandy loam	Casa Grande
S-20*	Gila silt loam	Welton-Mohawk Valley
S-30*	McAlister sandy clay loam	Willcox
S-40*	Gila silt loam	Salt River Valley
S-50*	Gila fine sandy loam	Casa Grande
S-60*	Cajon sandy loam	Casa Grande
S-80*	Gila silt loam	Salt River Valley
S-90*	Superstition sand	Yuma Mesa
S-100*	Gila silt loam	Cortaro
S-110**	Tucson sandy loam	Tucson
S-200 [†]	Karro silt loam	Kansas settlement

*Soils utilized by Jones (26).

**This soil was omitted from all analyses except the NH_4F and NH_4Cl versus Bray's P-1.

[†]Soil for which unpublished data were available.

taking the difference between the total phosphorus and inorganic phosphorus content for each particular soil.

Procedure for Extraction and Determination
of Phosphorus in Soil Fractions

The procedure used to obtain the phosphate fractions was the one proposed by Chang and Jackson (8), with a slight modification. The modification consisted of the addition of 0.1 gram of calcium acetate to the 0.5 N NaOH extracting solution. This modification was based on work performed by Dean (13). He showed that 0.25 N NaOH could dissolve from suspensions containing pure compounds of mono-, di-, and tricalcium phosphates (in the amount of 10 mgms. each) 73%, 28%, and 7% phosphorus, respectively. However, when 1 gm. of calcium acetate per 10 mgms. of phosphate was added to the solution the mono-, di-, and tricalcium phosphates were soluble at the rate of 0.5%, 0.2%, and 0.2% phosphorus, respectively. It was felt that the inclusion of calcium acetate would substantially reduce the dissolution of the calcium phosphates in the 0.5 N NaOH extraction solution of the Chang and Jackson procedure.

Another modification consisted of the omission of the dithionate-citrate extraction. Chang and Jackson (8) referred to this fraction as the reductant soluble

phosphorus. It was felt that this fraction would be negligible in its effect on the growth of plants since it represents only the difficultly soluble phosphorus. The amount of phosphorus in this fraction was not extracted and is therefore included in the fraction which is called the remainder.

The development of the color for the phosphorus determination was after Dengies and Dickman and Bray, Methods I and II, as outlined in Jackson (25). Method I was used for developing the color in the sodium hydroxide and sulfuric acid extractions and Method II was used for developing the color in the ammonium chloride and ammonium fluoride extracts. The stannous chloride reducing agent was made by dissolving 25 gms. of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 50 ml. of concentrated HCl. This was used as the stock solution and 1 ml. of stock solution was diluted to 20 ml. with 0.632 N HCl immediately before using. The stock solution was stored in a brown plastic container in the dark. It was removed only to take an aliquot for making the working solution.

Mechanical Analysis of the Soils

The mechanical analyses were by the hydrometer method with modification by Day (10). The soils were first treated with 30% hydrogen peroxide to eliminate the organic

matter. The sand, silt, and clay separates were found from calculations based on the 2 micron and 50 micron values. The results are given in table (4).

Statistical Analyses

The statistical analyses used were the testing of the difference between two means, after Steel and Torrie (39), and multiple regression analyses after Steel and Torrie and Snedecor (38). Also, linear regression, according to Steel and Torrie and curvilinear regression according to Snedecor were used in the analysis of the data.

TABLE 4.--Mechanical Analysis of the Soils Used.

Soil	Sand	Silt	Clay
	%	%	%
S-10	84.0	10.0	6.0
S-20	42.0	37.5	20.5
S-30	65.0	18.5	16.5
S-40	51.0	30.0	19.0
S-50	65.0	17.5	17.5
S-60	69.5	15.5	15.0
S-80	52.0	38.5	9.5
S-90	94.0	3.0	3.0
S-100	50.0	32.0	18.0
S-200	49.5	31.0	19.5

The mechanical analysis was done according to Day (10) on air-dried samples.

RESULTS AND DISCUSSION

Chemical Extraction of Soils by Metha's Procedure

Total phosphorus. In the extraction of total phosphorus it was found that an addition of 1 ml. of 72% perchloric alone in Metha's procedure was not enough to oxidize the organic matter present. Therefore, 3 ml. of concentrated nitric acid was added and an additional 1 ml. of 72% perchloric acid. Also, since the aliquot taken for analysis could not be evaporated to dryness it was found that the residual perchloric acid readily oxidized the amino-naphthol-sulfonic acid reagent when added in the usual small quantities. This was overcome by adding 1 ml. of the reducing agent to the final solution in which the color was to be developed.

The results of this determination may be seen in table (5). It is readily seen that the total phosphorus in the calcareous soils of Arizona is quite varied. The mean phosphorus content of the soils tested ranged from approximately 155 ppm. to approximately 1125 ppm. phosphorus. When these amounts are projected to an acre furrow slice basis it can be seen that the total amount of phosphorus on any of these soils is more than sufficient

TABLE 5.--The Results of Duplicate Analyses of Soil for Total, Inorganic and Organic Phosphorus and Tests of Significant Differences Between Duplicates.

Soil	Fractions						
	Total			Inorganic			Organic
	1	2	Mean	1	2	Mean	Mean
	ppm						
S-10	212.0	228.3	220.1	195.7	220.1	207.9	12.2
S-20	880.5	994.6	937.5	839.7	880.5	860.1	77.5
S-30	342.4	375.0	358.7	293.5	342.4	318.0	40.8
S-40	994.6	929.4	962.0	970.1	896.8	933.5	28.5
S-50	668.5	644.1	656.3	619.6	603.3	611.4	44.8
S-60	464.7	464.7	464.7	415.8	432.1	424.0	40.8
S-80	1076.1	1092.4	1084.3	994.6	1010.9	1002.8	81.5
S-90	138.6	171.2	154.9	126.4	171.2	148.8	6.1
S-100	1141.1	1108.7	1124.9	994.6	1027.2	1010.9	114.0
S-200	554.4	554.4	554.4	448.4	489.2	468.8	85.6
\bar{x}	647.3	656.3		589.8	607.4		
R_s	1524			1467			
t	< 1, N.S. at 0.05			< 1, N.S. at 0.05			

for any single crop. However, it is known that all of this phosphorus is not readily soluble and consequently usable by plants during a single growing season. This is precisely the reason, according to Bray (4), that other chemical methods were developed.

Inorganic phosphorus. The analysis of the soil extracts for inorganic phosphorus was very straightforward and required no adjustments of the procedure. In pipetting an aliquot for analysis care must be taken not to disturb the flocculated organic matter that has settled out of solution. The withdrawal of some of this organic matter may cause erroneously high results in the determination of the inorganic fraction.

The results of this extraction may be seen in table (5). The mean values for the inorganic fraction ranged from 149 ppm. to 1011 ppm. phosphorus. In all cases the inorganic fraction as determined by this method was less than the total phosphorus extracted. This indicates two very important facts: (1) that the method was reasonably precise, and (2) that all soils tested contained organic phosphorus to some extent.

Organic phosphorus. Organic phosphorus was determined by taking the difference between the mean total and mean inorganic phosphorus content of the soils. This fraction contained a surprising amount of phosphorus.

The results, shown in table (5), indicate that quite large amounts of organic phosphorus were found in these soils. The mean organic phosphorus content ranged from approximately 6 to 114 ppm. phosphorus. On a percentage basis the organic phosphorus accounted for as little as 3 percent to as high as 15.5 percent of the total phosphorus. These values are similar to values found by Sen Gupta and Cornfield (37) and lower than those reported by Fuller and McGeorge (19) when they studied organic phosphorus on calcareous soils. It is surprising to find as much phosphorus in the organic form in these calcareous soils which are notoriously low in organic matter based on organic carbon content. The organic matter content of Arizona soils generally varies from about 0.5 to 1.5 percent.

The organic phosphorus is probably found in soil in one of the following three forms: (1) as a constituent of phytin, (2) in the form of phosphate bound in coenzymes and phosphipids both in root residues and dead microbial cells, and (3) to some extent as exudates from living bacterial cells. The first two, no doubt, contribute by far the most phosphorus.

Statistical analysis. Since duplicate analyses were made on all the soils for each of the extracts the data were analyzed statistically to determine if the results from each set of analyses could be combined to obtain a

mean value for use in subsequent statistical treatment of the data. The method of analysis used was from Steel and Torrie (39).

As can be seen in table (5) no significant differences occurred between the first set of extractions and the second set for either the inorganic or total phosphorus fractions. Therefore, it was assumed that no significant difference occurred between the organic phosphorus duplicates.

In order to evaluate the relationship between these fractions and crop response some other statistical analyses were made. These consisted of linear regression and multiple regression after Steel and Torrie (39) and Snedecor (38). All the comparisons were made with phosphorus in the extract expressed as ppm. and yield from untreated soil expressed in grams of dry weight.

By inspection of the graphs of total and inorganic phosphorus versus yield it is apparent that with such a scattering of points no relationship existed between these fractions and yield. See figures (1) and (2).

The data for the organic fraction and yield shown in figure (3) indicates a slight possibility of some relationship existing between the two variables. A linear regression was made on the organic fraction versus the

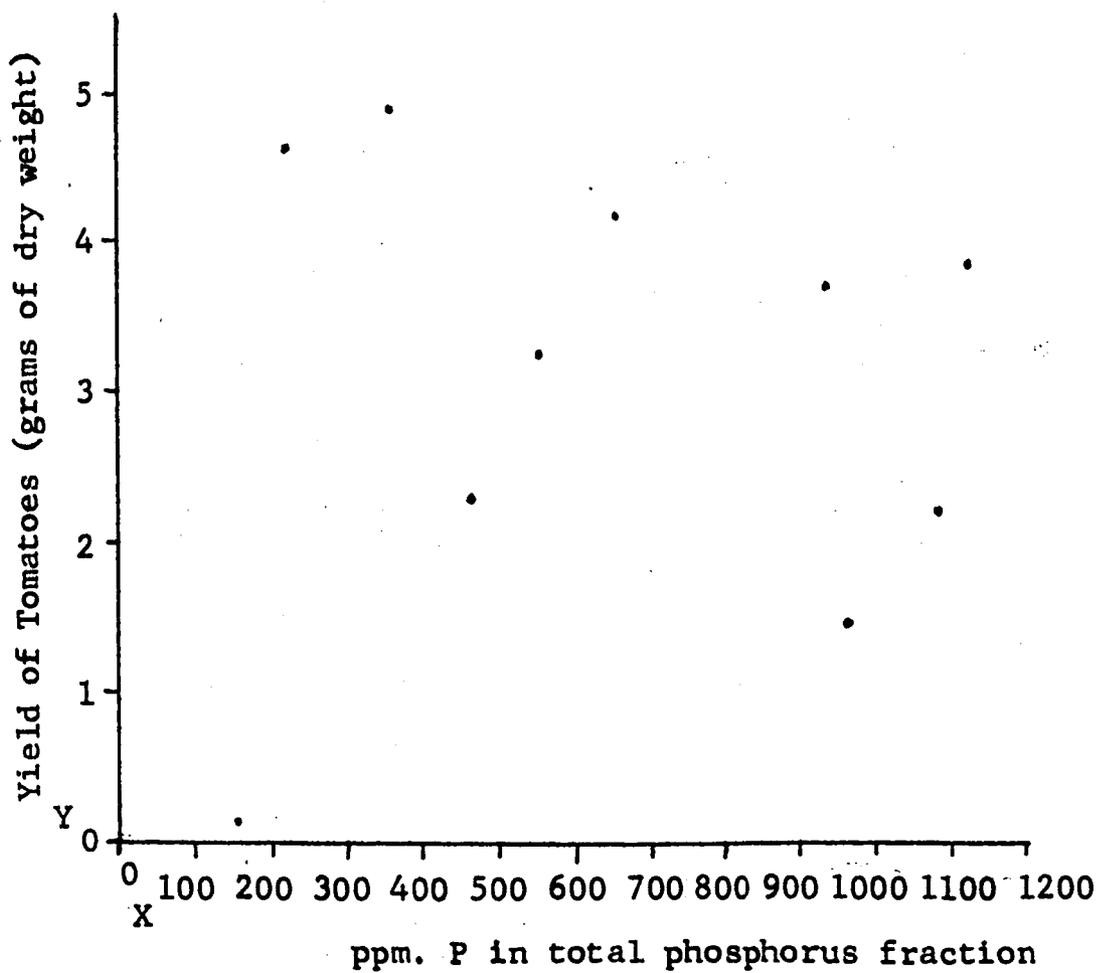


Figure 1.--Total Phosphorus as Determined by Metha's Procedure versus Yield of Tomatoes.

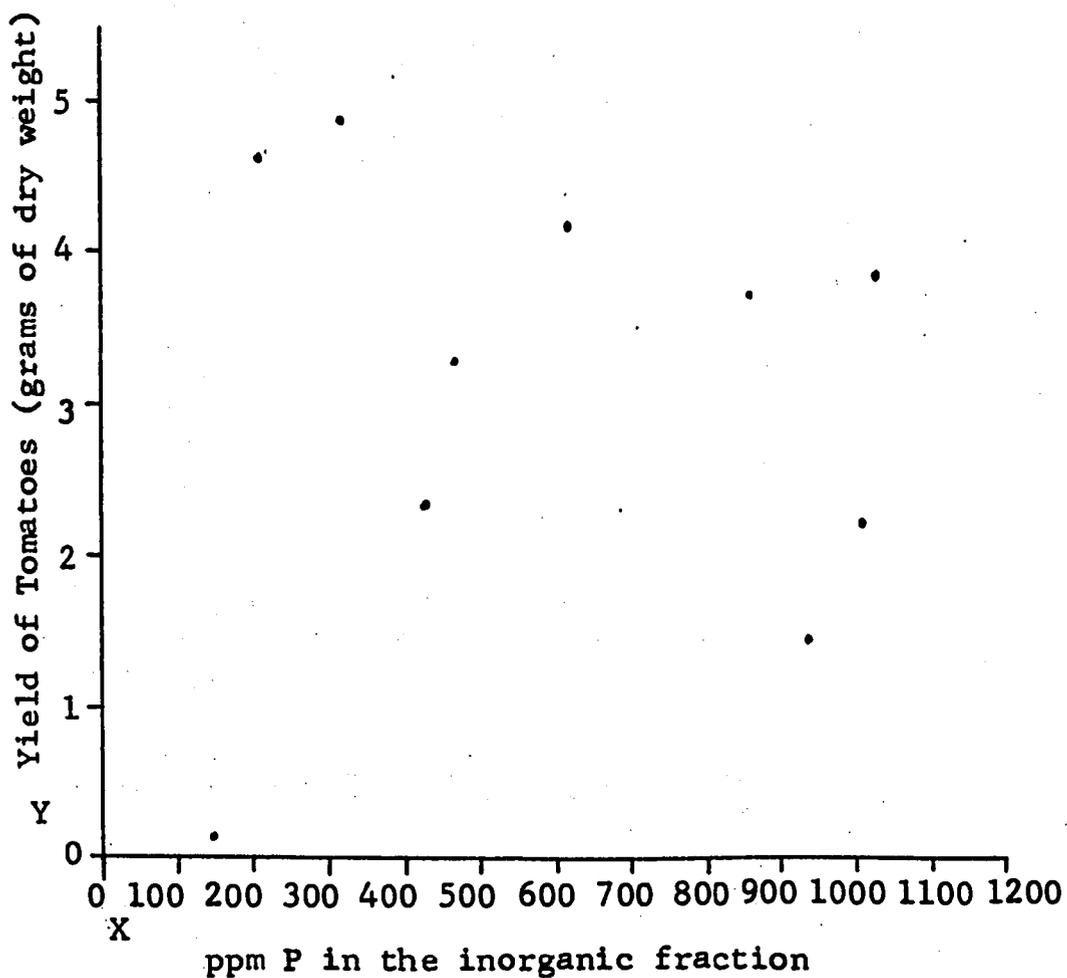


Figure 2.--Inorganic Phosphorus as Determined by Metha's Procedure versus Yield of Tomatoes.

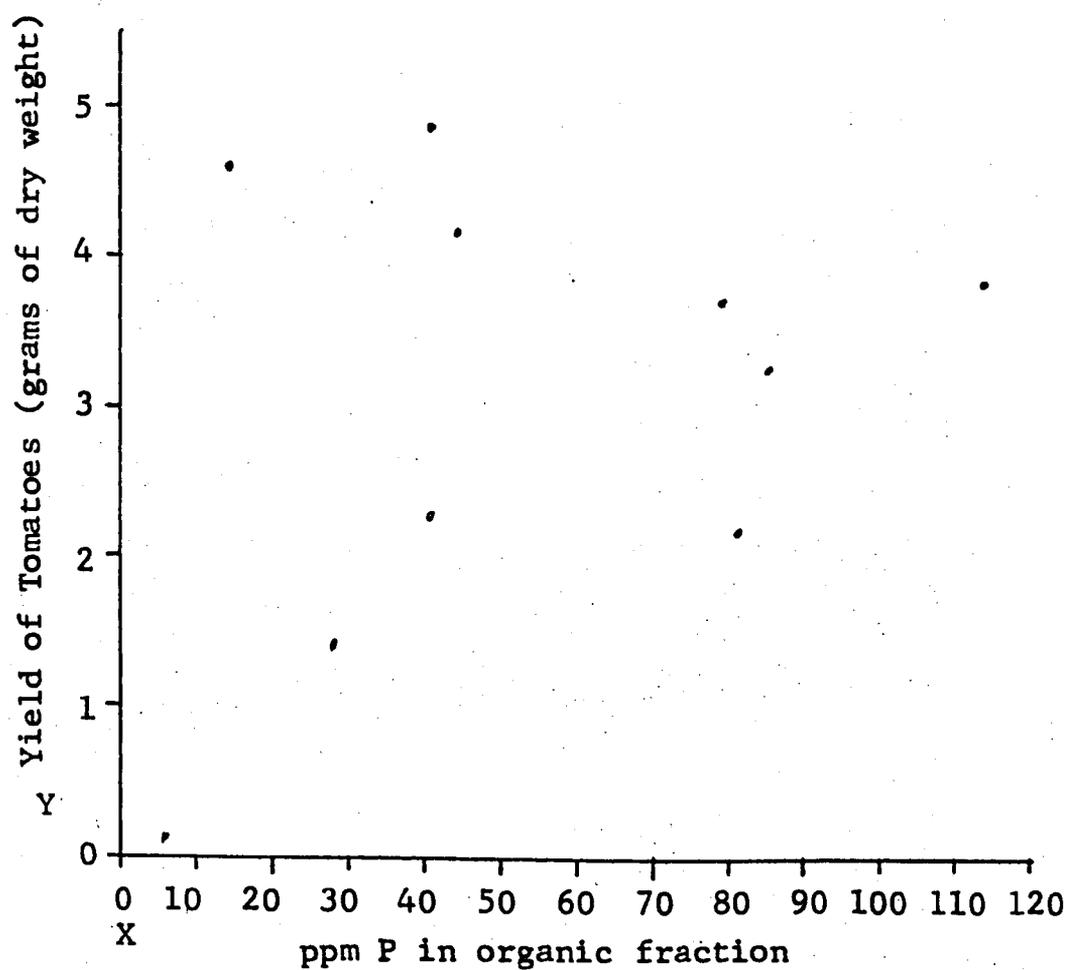


Figure 3.--Organic Phosphorus as Determined by Metha's Procedure versus Yield of Tomatoes.

yield and no significant correlation ($r = 0.287$) was found between the two variables.

After inspection of the graphical representation of the organic phosphorus and yield data it was felt that perhaps a curve in the shape of a parabola could fit the points. In order to fit this curve a multiple regression analysis was made with the two X variables represented by X and X^2 . This method of fitting a second degree polynomial is more commonly called curvilinear regression. The method employed was that of Snedecor (38).

The resultant multiple correlation coefficient of 0.354 is not significant. This again indicates that no relationship existed between the organic phosphorus found in the soil and the yield obtained on that soil.

The fact that no relationship existed between the total or inorganic phosphorus and yield was to be expected since in the past no investigator has found a significant relationship. It was felt that since a substantial amount of organic phosphorus was present it would contribute to crop growth, particularly in view of the rather high solubility of organic phosphorus in water as reported by Fuller and McGeorge (19). At any rate, the possibility of the organic phosphorus contributing to crop response should not be discounted. This should become apparent in a later discussion.

Chemical Extraction of Soil by the Chang
and Jackson Method

The calcium phosphate fraction. This fraction is removed from the soil sample by the action of 0.5 N sulfuric acid. It was the last fraction to be extracted in the analysis of the soil sample. The results of duplicate extractions are given in table (6).

There are several forms in which phosphorus may exist in combination with calcium. These forms are mono-, di-, and tricalcium phosphates, and apatites. The apatites include hydroxy-, fluoro-, chloro- and carbonato-phosphates. There is some doubt that the monocalcium phosphate does exist in calcareous soils. Since it is relatively water soluble it is believed that the monocalcium phosphate is soon reverted to some less soluble form. Since there is an abundance of calcium in the soil most of the monocalcium phosphate probably is reprecipitated as the less soluble di- and tricalcium phosphates. This does not preclude its reprecipitation as iron and aluminum phosphates which are even less water soluble than the di- and tricalcium phosphates.

The phosphate which remains undissolved after the sulfuric acid treatment is called the remainder. The forms of phosphorus in this fraction are principally the difficult to dissolve minerals of phosphorus such as

TABLE 6.--Phosphorus Fractionation of Duplicate Soil Samples by Chang and Jackson's Procedure.

Soil	Fractions										
	NH ₄ Cl			NH ₄ F			H ₂ SO ₄			Summation of Extracts	
	1	2	Mean	1	2	Mean	1	2	Mean	1	2
	ppm										
S-10	16.65	8.13	12.39	11.35	11.35	11.4	137.5	162.5	150.0	167.5	183.8
S-20	12.50	13.75	13.12	82.50	91.25	86.9	775.5	900.0	837.8	868.1	1004.4
S-30	8.13	10.60	9.36	25.00	30.00	27.5	212.5	225.0	218.8	245.0	264.4
S-40	5.63	5.63	5.63	35.00	25.63	30.3	900.0	900.0	900.0	940.0	930.6
S-50	11.88	11.88	11.88	57.50	55.63	56.6	512.0	562.5	537.3	580.8	629.4
S-60	3.12	4.38	3.75	21.25	25.00	23.1	320.0	350.0	335.0	341.3	375.0
S-80	8.13	7.50	7.81	41.90	30.00	36.0	862.5	887.5	875.0	911.3	924.4
S-90	5.00	5.63	5.31	6.25	7.50	6.9	187.5	150.0	168.8	193.8	162.5
S-100	25.60	28.10	26.85	157.50	207.50	182.5	812.5	650.0	731.3	995.0	885.0
S-200	7.50	6.90	7.20	21.90	26.90	24.4	162.5	500.0	331.3	190.6	533.8
\bar{x}	10.41	10.25		46.02	51.08		488.3	528.8		543.3	589.3
s_d	3.05			23.4			139.3			153.3	
t	< 1, N.S. at 0.05			2.165, N.S. at 0.05			2.91 at 0.05			< 1, N.S. at 0.05	

Iron phosphate fraction not detectable.

dufrenite, wavellite, lazulite, and vivanite. Also phosphates which are occluded in the crystalline structure of clay minerals are included in this fraction. These phosphates are generally considered to be rather permanently fixed and therefore of little consequence as far as supplying phosphates for plant nutrition.

Iron phosphate fraction. Phosphorus was not detectable in this fraction of the soil phosphates. This indicates that less than 2.50 ppm. phosphorus existed as iron phosphate in the soils tested since this amount was the lowest detectable by the procedure used.

Three possible explanations can be given for the inability to detect phosphate in these soils. First of all, the quantities of iron phosphate present may have been insufficient to detect. Secondly, since calcium acetate was added to the sodium hydroxide extracting solution it may have caused the reprecipitation of the dissolved iron phosphate as calcium phosphate during the extraction period. In view of the fact that other investigators (15, 27) also have reported little or no iron phosphate when using the Chang and Jackson procedure for fractionation it is probable that the lack of detection of iron phosphate was not due to the addition of calcium acetate. Finally, solublizing of the iron phosphate could have occurred during the previous extraction with ammonium

fluoride. However, several investigators (16, 29, 43) have studied the specificity of the ammonium fluoride extractant for the aluminum phosphates and have found that at alkaline pH values of 8.2 to 8.5 for the recommended extraction period of 1 hour only trace amounts of iron phosphate is removed. Therefore, in the author's opinion, this effect also may be discounted. Thus the first explanation appears to be more tenable.

Because of the relatively high solubility of the organic matter in the sodium hydroxide extractant the extract was treated with 1 ml. of concentrated sulfuric acid and allowed to stand for 2 hours. After this period the extract was examined for flocculated organic matter and if present the sample was centrifuged and the supernatant liquid removed. This procedure reduced any possibility of organic phosphorus being present in the analysis for iron phosphate.

Aluminum phosphate fraction. The results of the extraction of duplicate soil samples for aluminum phosphate may be seen in table (6). The mean values for phosphorus in this fraction varied from a low of approximately 7 ppm. to a high of approximately 183 ppm. The possibility that this fraction contains some other form of soil phosphorus does exist and therefore must be evaluated.

The presence of iron phosphate, as discussed previously, is only very slight and can be considered negligible. However, there are three other forms of soil phosphates which could contribute to the phosphorus found in this fraction. These are the calcium phosphates, organic, and adsorbed phosphates.

The amount of calcium phosphate dissolved by the ammonium fluoride extractant was found by Glenn et al. (22) to be negligible when they extracted natural soil containing large amounts of calcium phosphate.

Quantitative or qualitative data are not available to the knowledge of the author concerning the solubility of the organic phosphates in the ammonium fluoride extractant. One might suppose that some solubilizing of the organic phosphates would result in this extraction but one can only speculate as to the quantities.

By examining the summation of all the extracts from the Chang and Jackson procedure as seen in table (6) and comparing this value with the amount of phosphorus found in the inorganic fraction from Metha's procedure as seen in table (5) it can be seen that three soils (S-20, S-40, and S-90) contain more phosphorus in their sums of the extracts than is found in the inorganic fraction by Metha's procedure. This could be due in part to the dissolving of the organic fraction by the ammonium

fluoride. In several soils the ammonium fluoride extractable phosphorus was lower than the organic phosphorus fraction. This would indicate that only a small part of the organic phosphorus was dissolved by the ammonium fluoride but the precise amount can not be ascertained.

The existence of adsorbed phosphates which can contribute to the phosphorus in the ammonium fluoride extract also is possible. Dean and Rubins (11) studied the amounts of exchangeable phosphorus in an anion exchange system. They found a definite correlation between anion exchange capacity and clay content of the soil. The amounts of exchangeable phosphorus that could be detected were quite high for the acid soils that they used. The fluoride ion was found capable of removing all the adsorbed phosphorus in soils with low anion exchange capacity and almost all the adsorbed phosphorus in soils with high anion exchange capacity. Therefore, one can conclude that if any adsorbed phosphorus existed on the clay of the soils tested in this experiment it was removed. The amount of phosphorus in this form is not known and can not be distinguished easily from the aluminum phosphate form.

Ammonium chloride soluble fraction. This fraction is referred to as the easily soluble and saloid bound phosphorus. The results of this extraction are given in table

(6). The mean phosphorus content in this fraction ranged from approximately 4 ppm. to approximately 27 ppm.

The forms of soil phosphorus which are extracted by this solution are not discernible. In all probability it extracts a slight amount of phosphorus from each of the main fractions. One would expect the highly water soluble organic phosphates to contribute to the amount of phosphorus found in this fraction. Also, those phosphates which have a large specific surface such as iron and aluminum phosphate may be found in the extraction. The di- and tricalcium phosphates are slightly soluble in water and with the quantity present in these soils a measurable amount of phosphorus might be dissolved in the ammonium chloride fraction. Another component which could be contributing to the phosphorus in the ammonium chloride fraction is the phosphorus bridged with some cation, most likely calcium in these soils, to the clay minerals present in the soil. It is felt by some workers that this is one of the ways in which phosphorus is adsorbed in soils. If indeed this is true the action of the ammonium ion would replace the adsorbed cation and free the phosphate.

Statistical Analyses of the Fractions

Testing of difference between two means for each fraction. Each of the fractions were tested to ascertain

if significant differences occurred between duplicate analyses. The results of the analyses are presented in table (6). Significant differences were not found between the duplicate samples in the ammonium chloride and ammonium fluoride fractions. However, there was a significant difference between the duplicate analyses for the sulfuric acid extraction. The difference between the duplicates of the sulfuric acid extraction may be attributed to one of two possibilities: first, that a difference actually did exist between the duplicate samples, and secondly, that in rejecting the null hypothesis a type I error was committed and a true difference did not exist between the two means.

In order to assess the latter possibility a test of the difference between two means was made on the summation of all the extracts. The results in table (6) show that no significant difference existed between the duplicate analyses with regard to the total of all the extracts. Because of this and since the values are commensurate with the values for the inorganic fraction as determined by Metha's procedure it was concluded that a type I error had been committed. Therefore, the mean values of the duplicates for the sulfuric acid extraction were used in subsequent statistical analyses.

Correlations of the calcium phosphate fraction with yield. From an inspection of the data in figure (4) it

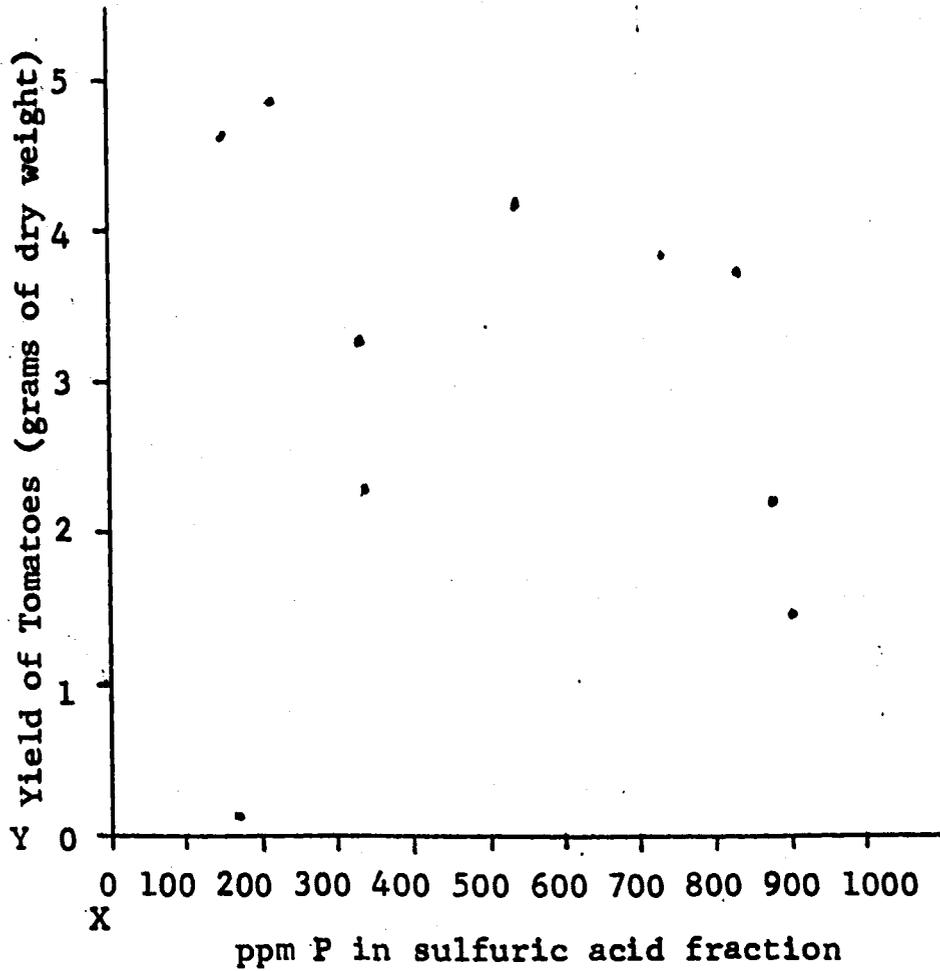


Figure 4.--Phosphorus in Sulfuric Acid Extract of Chang and Jackson Procedure versus Yield of Tomatoes.

appears that a linear relationship existed between the amounts of phosphorus from 150 to 600 ppm. in the calcium phosphate fraction and actual yield of tomatoes. It appears, also, that a linear decrease in yield occurred at levels of calcium phosphate greater than approximately 600 ppm.

A curvilinear analysis was made using the method of Snedecor (38). The mean values obtained for the calcium phosphate fraction in ppm. were used in the analysis. The variables were Ca-PO_4 and $(\text{Ca-PO}_4)^2$ versus yield. The correlation coefficient obtained for this analysis was 0.303 and was not significant. This indicated that only 9 percent of the variation in yield was associated with the variation in the amounts of calcium phosphate.

Correlations of aluminum phosphate with yield.

The data shown in figure (5) indicates a possible linear relationship between the ammonium fluoride extractable phosphorus and yield. A linear regression analysis using the method of Steel and Torrie (39) was made with the aluminum phosphate fraction expressed in ppm. phosphorus and yield of tomatoes expressed as grams oven dry weight.

The results of this analysis shown in table (7) indicate no significant linear relationship between the two variables. It was felt that the relationship might be improved if the point most distant from the others was removed and a similar analysis made. However, the

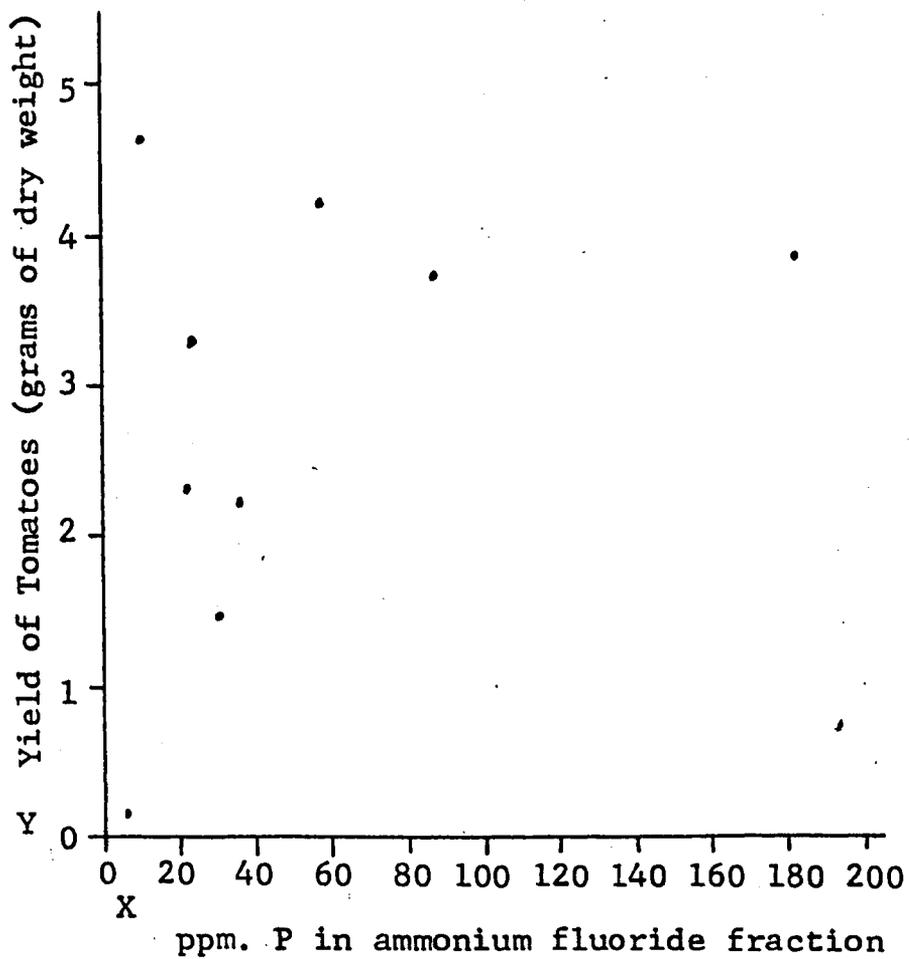


Figure 5.--Phosphorus in Ammonium Fluoride Extract of Chang and Jackson Procedure versus Yield of Tomatoes.

TABLE 7.--The Results of Regression Analyses of Ammonium Fluoride Fraction and Yield of Tomatoes.

Variables	Correlation Coefficient
Linear Regression	
NH ₄ F fraction <u>vs.</u> yield	r = 0.30, N.S. at 0.05
NH ₄ F fraction <u>vs.</u> yield (S-100 omitted)	r = 0.33, N.S. at 0.05
Curvilinear Regression	
NH ₄ F and (NH ₄ F) ² <u>vs.</u> yield	R = 0.42, N.S. at 0.05

Note: significant r at $\alpha = 0.05$ with 8 degrees of freedom = 0.632. Significant r at $\alpha = 0.05$ with 7 degrees of freedom = 0.666. Significant R at $\alpha = 0.05$ with 2 variables, 7 degrees of freedom = 0.758.

removal of soil S-100 from the analysis increased the value of the correlation coefficient only very slightly. See table (7). Apparently there is no linear correlation of aluminum phosphate with yield at all.

In order to investigate the possibility of a curvilinear relationship, a multiple regression analysis was made using X and X^2 with aluminum phosphate as the X variable. The result of this analysis shown in table (7) also indicates that no significant curvilinear relationship existed between the aluminum phosphate fraction and yield. Thus it appears that aluminum phosphate is in no way significantly associated with yield.

Correlation of the ammonium chloride fraction with yield. The data shown in figure (6) indicates that a possibility of a linear relationship exists. A linear regression analysis was performed on the phosphorus in the ammonium chloride fraction versus yield. It was found that the linear correlation coefficient was not significant. However, if the point corresponding to soil S-100 is removed from the analysis a correlation coefficient of 0.754 is found which is significant at the 0.05 level. The results of all correlations and the analysis of variance of regression are reported in table (8). The significant F value at 0.05 indicates also that the correlation coefficient is significant.

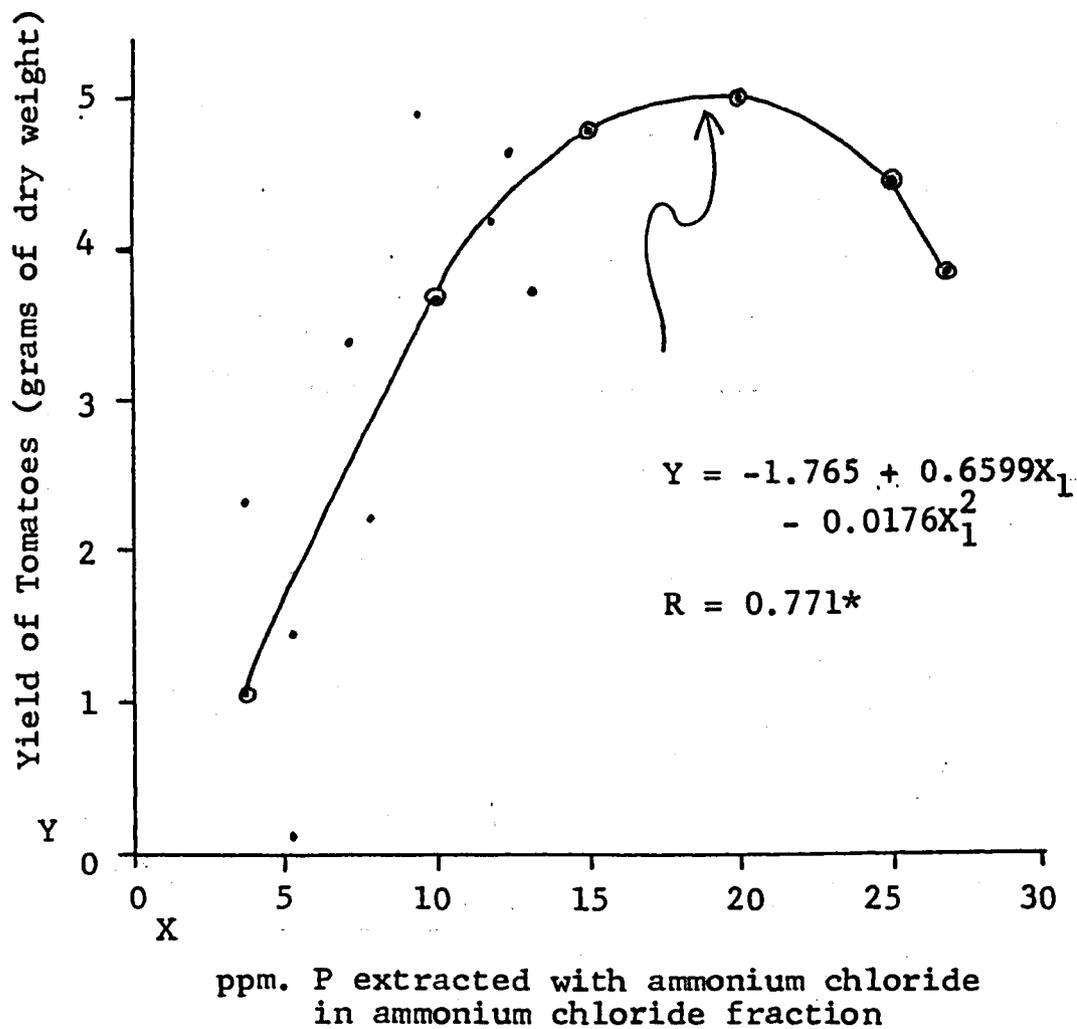


Figure 6.--Regression of Yield of Tomatoes on Phosphorus in the Ammonium Chloride Extract of the Chang and Jackson Procedure.

TABLE 8.--The Results of Linear Regression Analyses with Ammonium Chloride Fraction versus Yield of Tomatoes.

Variables	Correlation Coefficient			
Linear Regression ^a				
NH ₄ Cl vs. yield	r = 0.52, N.S. at 0.05			
NH ₄ Cl vs. yield (S-100 omitted)	r = 0.754* at 0.05			
Source	df	ss	ms	f _{calc.}
<u>Analysis of Variance (NH₄Cl vs. yield, S-100 omitted)^b</u>				
Regression ss	1	11.4357	11.4357	9.202*
Deviation ss	7	8.6987	1.2427	
TOTAL	8	20.1344		
<u>Analysis of Variance (NH₄Cl vs. yield)^c</u>				
Regression ss	1	5.5371	5.5371	2.899, N.S.
Deviation ss	8	15.2786	1.9100	
TOTAL	9	20.8157		

^a Significant r at $\alpha = 0.05$ and 8 degrees of freedom = 0.632. Significant r at $\alpha = 0.05$ and 7 degrees of freedom = 0.666.

^b Tabular F at $\alpha = 0.05$ with 1 and 7 degrees of freedom = 5.59

^c Tabular F at $\alpha = 0.05$ with 1 and 8 degrees of freedom = 5.32.

A valid reason can not be given for the deletion of the point corresponding to soil S-100 from the analysis. Each of the soils was treated alike in the chemical analysis and no discernible feature about this soil could be identified which would distinguish it from the other soils. Nevertheless, soil S-100 must possess some physical or chemical characteristics that influenced yield or the solubility of phosphorus in the ammonium chloride in a manner disproportionate to the other soils.

A curvilinear analysis was performed which included the ten soils. The analysis was similar to those previously made. The X variable was the phosphorus in the ammonium chloride fraction expressed as ppm. and the Y variable was yield in grams of oven dry materials. A significant curvilinear relationship was found. The multiple regression coefficient was 0.771 which is significant at the 0.05 level. The results of this analysis may be seen in table (9).

In order to observe the effect of curvilinearity of regression on the deviations from regression an analysis of variance was made to test the significance of departure or curvilinear regression from linear regression. The analysis of variance and test of significance are shown in table (9). The reduction in the sum of squares, tested against the mean square remaining after curvilinear regression, was significant. Therefore, the hypothesis of linear

TABLE 9.--The Results of Curvilinear Regression with NH_4Cl and $(\text{NH}_4\text{Cl})^2$ versus Yield of Tomatoes.

Variables	Correlation Coefficient			
Curvilinear Regression ^a				
NH_4Cl and $(\text{NH}_4\text{Cl})^2$ vs. yield	R = 0.771* at 0.05			
Source	df	ss	ms	f _{calc.}
Analysis of Variance [NH_4Cl and $(\text{NH}_4\text{Cl})^2$ vs. yield] ^b				
Regression ss	2	12.4824	6.2412	5.243*
Deviation ss	7	8.3333	1.1905	
TOTAL	9	20.8157		
Analysis of Variance for the test of significance of departure from linear regression ^c				
Deviation from linear regression	8	15.2786		
Deviation from curvilinear regression	7	8.3333	1.1905	
Curvilinearity of regression	1	6.9453		5.83,* 0.05

^aSignificant R at $\alpha = 0.05$ with 2 variables and 7 degrees of freedom = 0.758.

^bTabular F at $\alpha = 0.05$ with 2 and 7 degrees of freedom = 4.74.

^cTabular F at $\alpha = 0.05$ with 1 and 7 degrees of freedom = 5.59.

regression is abandoned. The prediction equation was calculated according to Snedecor (38) and is shown in figure (6).

The fitting of the points to a parabola would indicate that as the phosphorus content in the ammonium chloride extract increased, the yield of tomatoes increased to a certain point. However, after that point was reached the yield would decrease for each added increment of native phosphorus. One might assume that the addition of phosphorus would decrease yield when applied to a soil with this level of ammonium chloride extractable phosphorus. The application of phosphorus to soil S-100 increased the yield significantly. Therefore, it appears that, although these data fit a parabolic curve, the curve is unrealistic in terms of plant behavior at the higher levels of ammonium chloride extractable phosphorus. Since a linear relationship existed between yield and the ammonium chloride extractable phosphorus when soil S-100 was deleted from the analysis, it appears that data for this soil is responsible for the parabolic character of the curve.

Linear, multiple, and curvilinear correlation with various combinations of the fractions and yield. Several analyses were made to study the relationships between yield and the amount of phosphorus in various combinations of fractions. Linear regression analyses were

performed between yield and the following combination of extracts: the sum of the ammonium chloride and ammonium fluoride extractable phosphorus, the sum of the ammonium chloride and organic fractions, and the sum of the ammonium fluoride, ammonium chloride, and organic fractions. The results of these analyses are shown in table (10). In no case were any of the correlation coefficients significant.

A linear regression also was performed with the phosphorus in the ammonium chloride fraction divided by the percent clay present in the soils versus yield. This relationship was studied since it has been reported by Pratt (34) that the ammonium chloride plus ammonium fluoride extractable phosphorus expressed as ppm. clay was related to the phosphorus in Bray's P-1 extract. The result of this analysis shown in table (10) shows the relationship was not significant.

Jones (26) analyzed ten soils for phosphorus by extracting them with Bray's P-1 extracting solution. When the amount of phosphorus found in the ammonium chloride fraction was statistically fitted to Mitscherlich's equation, according to Snedecor (38), a highly significant correlation coefficient of 0.952 was found. The ammonium fluoride extractable phosphorus when analyzed in the same manner also was found to be highly correlated ($r = 0.882$). In both cases the phosphorus was expressed as phosphorus in ppm. of soil.

TABLE 10.--The Results of Linear Regression and Several Fractions Alone and in Various Combinations versus Yield of Tomatoes.

Variables	Correlation Coefficient
$\text{NH}_4\text{Cl} + \text{NH}_4\text{F}$ <u>vs.</u> yield	$r = 0.33$, N.S. at 0.05
$\text{NH}_4\text{Cl} + \text{organic}$ <u>vs.</u> yield	$r = 0.32$, N.S. at 0.05
$\text{NH}_4\text{Cl} \div \% \text{Clay}$ <u>vs.</u> yield	$r = .0001$, N.S. at 0.05
$\text{NH}_4\text{Cl} + \text{NH}_4\text{F}$ <u>vs.</u> yield (S-100 omitted)	$r = 0.43$, N.S. at 0.05

Note: Significant r at $\alpha = 0.05$ with 8 degrees of freedom = 0.632. Significant r at $\alpha = 0.05$ with 7 degrees of freedom = 0.666.

Since only approximately 50 percent of the variation in yield was associated with the variation in phosphorus in the ammonium chloride fraction it was felt this fraction and some other fraction could be significantly associated with yield in a multiple regression analysis.

Three separate multiple regressions were made with the X_2 variable represented by the following: the ammonium fluoride fraction, the organic fraction, and the percent clay in the soil. The results in table (11) indicate that none of the relationships were significant.

Several curvilinear regressions also were performed using the summation of two or more fractions as the X variable. The results given in table (11) show that none of these analyses were significant.

TABLE 11.--The Results of Multiple and Corvilinear Regression Analyses with the Fractions in Various Combinations versus Yield.

Variables	Correlation Coefficient
Multiple Regression	
NH ₄ Cl and NH ₄ F <u>vs.</u> yield	R = 0.69, N.S. at 0.05
NH ₄ Cl and % clay <u>vs.</u> yield	R = 0.58, N.S. at 0.05
NH ₄ Cl and organic <u>vs.</u> yield	R = 0.51, N.S. at 0.05
Curvilinear Regression	
(NH ₄ Cl + organic) and (NH ₄ Cl + organic) ² <u>vs.</u> yield	R = 0.58, N.S. at 0.05
(NH ₄ Cl + NH ₄ F + organic) and (NH ₄ Cl + NH ₄ F + organic) ² <u>vs.</u> yield	R = 0.35, N.S. at 0.05
(NH ₄ Cl + NH ₄ F) and (NH ₄ Cl + NH ₄ F) ² <u>vs.</u> yield	R = 0.47, N.S. at 0.05
(NH ₄ F + organic) and (NH ₄ F + organic) ² <u>vs.</u> yield	R = 0.37, N.S. at 0.05
NH ₄ Cl and (NH ₄ Cl) ² <u>vs.</u> yield (S-100 omitted)	R = 0.69, N.S. at 0.05

Note: Significant R at $\alpha = 0.05$ with 2 variables and 6 degrees of freedom = 0.795.

SUMMARY AND CONCLUSIONS

A laboratory study was conducted to characterize the soil phosphorus of ten agriculturally important soils of Arizona. Information on these soils concerning yield of tomato plants in the greenhouse and other factors was available.

The laboratory analyses consisted of extracting the phosphorus from these soils by two methods:

1. Metha's procedure which consisted of the following fractions:
 - a. total phosphorus
 - b. inorganic phosphorus
 - c. organic phosphorus (by difference)
2. Chang and Jackson's procedure which consisted of the following fractions:
 - a. ammonium chloride extractable phosphorus
 - b. ammonium fluoride extractable phosphorus
 - c. sodium hydroxide extractable phosphorus which included the addition of 0.1 gm. of calcium acetate
 - d. sulfuric acid extractable phosphorus
 - e. the remainder

A significant curvilinear regression coefficient of 0.771 was found between the ammonium chloride extractable soil phosphorus and yield of tomatoes. Also, a significant linear regression coefficient of 0.754 was found between the ammonium chloride extractable phosphorus versus yield of tomatoes when soil S-100 was omitted from the analysis. It is apparent that soil S-100 has some properties which are different from the other soils that influenced the results. These properties were not discernible.

It is of importance to consider that the ammonium chloride extractable phosphorus represents the easily soluble soil phosphorus. Since this fraction is associated with yield, it appears that one or more of the soil phosphate fractions are contributing to the phosphorus nutrition of the plant. The utilization of the discrete forms of soil phosphorus by plants did not appear to be proportionate for all soils. From this, one could conclude that the amount of phosphorus a fraction can contribute to the plant depends on the forms as well as amounts of the constituents in that fraction. For example, the calcium phosphate fraction contains di- and tricalcium phosphates and apatites, each of which has a different solubility.

More investigation is needed in this area to better understand the contributions of the discrete forms of soil phosphates to the phosphorus nutrition of the plant.

BIBLIOGRAPHY

1. Barrow, N. J. "Phosphorus in Soil Organic Matter," Soils & Fertilizers No. 3, 24:169-163 (1961).
2. Bhangoo, M. S. and Smith, F. W. "Fractionation of Phosphorus in Kansas Soils and its Significance in Response of Wheat to Phosphate Fertilizers," Agron. J. No. 749:354-358 (1957).
3. Bray, R. H. "New Concepts in the Chemistry of Soil Fertility," S. S. S. A. P., 2:175-179 (1937).
4. Bray, R. H. "Requirements for a Successful Soil Test," Soil Sci., No. 2, 66:83-89 (1948).
5. Breaszeale, J. F. and Burgess, P. S. "The Availability of Phosphates in Calcareous or Alkaline Soils," Ariz. Agr. Expt. Stn. Technical Bulletin No. 10, 1 June 1926.
6. Chai Moo Cho and Caldwell, A. C. "Forms of Phosphorus and Fixation in Soils," S. S. S. A. P. No. 6, 23:458-460 (1960).
7. Chang, S. C. and Jackson, N. L. "Soil Phosphorus Fractions in Some Representative Soils," J. Soil Sci. No. 1, 9:109-119 (1958).
8. Chang, S. E. and Jackson, N. L. "Fractionating of Soil Phosphorus," Soil Sci. No. 2, 84:133-144 (1957).
9. Chang, S. C. and Juo, S. R. "Available Phosphorus in Relation to Forms of Phosphates in Soils," Soil Sci. No. 2, 95:91-96 (1958).
10. Day, Paul R. "Experimental Conformation of Hydrometer Theory," Soil Sci. 74:181-186 (1953).
11. Dean, L. A. and Rubins, E. J. "Anion Exchange in Soils; I Exchangeable Phosphorus and the Anion Exchange Capacity," Soil Sci. No. 5, 63:377-387 (1947).
12. Dean, L. A. "An Attempted Fractionation of Soil Phosphorus," J. Agri. Sci. 28:234 (1938).

13. Dean, L. A. "The Distribution of the Forms of Soil Phosphorus," S. S. S., A. P.; 2:223 (1937).
14. Dickman and Bray. "Colorimetric Determination of Phosphate," Ind. Eng. Chem. Analyt. Ed. 12:665 (1940).
15. El Bagouri, I. H. M. "The Effect of Soil Carbonate on the Availability of Added and Native Phosphorus in Some Calcareous Soils," Masters Thesis, Univ. of Manitoba, May 1962.
16. Fife, C. V. "An Evaluation of Ammonium Fluoride as a Selective Extractant for Aluminum Bound Soil Phosphate: III Detailed Studies on Selected Soils," Soil Sci. No. 2, 93:104-112 (1962).
17. Fisher, R. A. and Thomas, R. D. "The Determination of the Forms of Inorganic Phosphorus in Soils," J. Amer. Soc. Agron., 27:863 (1935).
18. Fraps, G. S. and Fudge, J. F. "The Nature of the Phosphates Dissolved by Various Soil Extractants," J. Amer. Soc. Agron. No. 5, 37:532-541 (1945).
19. Fuller, W. H. and McGeorge, W. T. "Phosphates in Calcareous Arizona Soils: II Organic Phosphorus Content," Soil Sci. No. 1, 71:45-50 (1951).
20. Ghani, M. O. "Fractionation of Soil Phosphorus: I Method of Extraction," Ind. J. Agri. Sci. Part 1, 13:29-45 (1943).
21. Ghani, M. O. "The Use of 8-Hydroxy Quinoline as a Means of Blocking Active Iron and Aluminum in the Determination of Available Phosphoric Acid of Soils by Dilute Acid Extractions," *ibid.*, 562-565 (1943).
22. Glenn, R. C., Hsu, P. H., Jackson, M. L., and Corey, R. B. "Flow Sheet for Phosphate Fractionation," Agronomy Abstracts 1959 Annual Meeting of Amer. Soc. of Agron., p. 9.
23. Hance, R. J. and Anderson, G. "A Comparative Study of Methods of Estimating Soil Organic Phosphate," J. Soil Sci. No. 2, 13:225-230 (1962).
24. Hus, P. H., Glenn, R. C., Corey, R. B., and Jackson, M. L. "Chemical Separation of Aluminum and Ferric Phosphates with Fluoride Solution," Agronomy Abstracts, 1960 Annual Meeting of Amer. Soc. Agron., p. 7.

25. Jackson, M. L. "Soil Chemical Analysis," Prentice-Hall, Inc., Englewood, New Jersey (1960).
26. Jones, P. J. "Chemical Methods for Evaluating Available Phosphorus in Arizona Soils," Masters Thesis, Univ. of Arizona, May 1959.
27. Lavery, J. C. and McLean, E. O. "Factors Affecting Yields and Uptake of Phosphorus by Different Crops: 3 Kinds of Phosphate--Native, Applied, and Formed," *Soil Sci.*, 91:166-171 (1961).
28. McGeorge, W. T. and Breazeale, J. F. "Phosphate Solubility Studies on Some Unproductive Calcareous Soils," Univ. of Ariz. College of Agr., Agricultural Expt. Stn., Technical Bulletin No. 35 (1931).
29. McGeorge, W. T. and Breazeale, J. F. "The Relation of Phosphate Availability, Soil Permeability, and Carbon Dioxide to the Fertility of Calcareous Soils," ibid., No. 36 (1931).
30. McGeorge, W. T. and Breazeale, J. F. "Studies on Iron and Aluminum and Organic Phosphates and Phosphate Fixation in Calcareous Soils," ibid., No. 40 (1931).
31. Metha, N. C., Legg, J. O., Goring, C. A., Black, C. A. "Determination of Organic Phosphorus in Soils: I Extraction Method," S. S. S. A. P. No. 4, 18:443-449 (1954).
32. Morgan, M. F. "Chemical Soil Diagnosis by the Universal Soil Testing System," Conn. Agr. Expt. Stn. Bulletin No. 450 (1941).
33. Patel, J. M. and Metha, N. C. "Soil Phosphorus Fractionation Studies," S. S. S. A. P. No. 3, 25:190-192 (1961).
34. Pratt, P. F. "Forms of Phosphorus in California Soils," Summaries of Papers Statewide Conference on Role of Phosphorus in Crop Production, Univ. of California, Davis, California, Jan. 1962.
35. Schofield, R. K. "Can a Precise Meaning be Given to 'Available' Soil Phosphorus," *Soils and Fertilizers*, No. 5, 18:373-375 (1955).
36. Sen Gupta, M. B. and Cornfield, A. H. "Phosphorus in Calcareous Soils: I The Inorganic Phosphate Fractions and Their Relation to the Amount of Calcium Carbonate Present," *J. Sci. Food Agr.*, No. 12, 13:651-655 (1962).

37. Sen Gupta, M. B. and Cornfield, A. H. "Phosphorus in Calcareous Soils: II Determination of the Organic Phosphorus Content of Calcareous Soils and Its Relation to Soil Calcium Carbonate Content," ibid., 655-658 (1962).
38. Snedecor, George W. "Statistical Methods," Iowa State College Press, Ames, Iowa (1959).
39. Steel, R. G. O. and Torrie, J. H. "Principles and Procedures of Statistics," McGraw-Hill Book Co., Inc., New York (1960).
40. Thompson, L. F. and Pratt, P. F. "Solubility of Phosphorus in Chemical Extractants as Indexes to Available Phosphorus in Ohio Soils," S. S. S. A. P., No. 4, 18:465-470 (1954).
41. Truog, Emil. "The Determination of Readily Available Phosphorus in Soils," J. Amer. Soc. Agron. 22:874 (1930).
42. Turner, R. C. and Rice, H. M. "Role of the Fluoride Ion in Release of Phosphate Adsorbed by Aluminum and Iron Hydroxides," Soil Sci. No. 2, 74:141-148 (1952).
43. Wada, K. and Jackson, M. L. "Chemical Kinetic Method for Separation of Aluminum Phosphate from Ferric Phosphate of Soils in Ammonium Fluoride," Agronomy Abstracts, 1961 annual meeting Amer. Soc. Agron., p. 15.
44. Williams, Colin H. "Studies on Soil Phosphorus I. A Method for the Partial Fractionation of Soil Phosphorus," J. Agr. Sci., Part 3, 40:233-242 (1950).
45. Williams, Rice. "The Solubility of Soil Phosphorus and Other Phosphorus Compounds in Sodium Hydroxide Solutions," J. Agr. Sci., 27:259 (1937).
46. Williams, R. J. B. and Cooke, G. W. "Measuring Soluble Phosphorus in Soils Comparisons of Methods and Interpretation of Results," J. Agri. Sci., 59:275-280 (1962).