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The Effect of Chelates on Phosphorus Availability and Mobility

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THE EFFECT OF CHELATES ON PHOSPHORUS AVAILABILITY AND MOBILITY

by

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of the requirements for the degree

of

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The thoughts with which I handled this problem were greatly affected by many people who gave me the best to be given--knowledge. To these people, whom I will ever remember with respect and love, I dedicate this work.

Salah Ahmed Tahoun

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INTRODUCTION

Soil-phosphorus relations and plant-phosphorus relations have attracted the interest of many investigators since Liebig introduced his famous theory about the importance of the mineral matters to the plant in 1840. It was soon realized that phosphorus nutrition was a problem not easily solved for two reasons. 1. The added phosphorus fertilizers, soon after soil application, are converted by some reactions in the soil to complex compounds far less soluble, consequently less available to the plant. Conclusions about this process led to controversial debates until it was discovered that a general statement covering all soils was impossible since the reactions involved in each case are different. 2. There was confusion caused by the use of two terms coined to express the plant's need for phosphorus. The first term, soluble phosphorus, was based on the assumption that nutrient absorption is a simple diffusion of ions from the soil solution into the roots while the second term, available phosphorus, was based on the observation that the plant absorbed more than that which could be estimated as soluble phosphorus from some insoluble sources. A general definition was given to the term available phosphorus as "that part of soil phosphorus which may be absorbed by ordinary crop plant in the production of plant substance." Later some restrictions were applied to regard the physical conditions of both soils and plants.

These two items just mentioned have an interaction which appears very clearly when the term available phosphorus is regarded from the point of determination rather than definition.

As mentioned above, the added fertilizer phosphorus is converted by soil reactions to less soluble complex compounds. The particular compound or compounds formed depend on the various soil factors. For example, in soils of intermediate to high pH, 6.0 to 9.0, the soluble phosphorus is ultimately converted to some form of apatite. The particular apatite formed, and, consequently, the degree of solubility is determined by other ions or compounds such as calcium carbonate ($CaCO_3$) or fluoride present in the soil. In soils of low pH, 5.0 and lower, the insoluble phosphorus compounds may be formed from iron or aluminum usually present. Such minerals as dufrenite, an iron containing one, and wavellite, an aluminum containing one, are considered insoluble. The applied phosphorus may be temporarily inactivated on the surface of the hydrated iron and aluminum oxides that may form in the acid soils.

The first attempts to get a chemical solvent that would extract the nutrient phosphorus from the soil in the same magnitude as extracted by plant roots was by the British scientist, Dyer (1894). He determined the root sap acidity of many different plant species and announced that 1.0 percent citric acid has an acidity which resembles to a great extent the acidity of roots. But this idea did not introduce any progress in phosphorus nutrition.

Of the many other solvents tried, the two used most were 0.2 $\underline{N}$ nitric acid (HNO_3) and 0.002 $\underline{N}$ sulfuric acid (H_2SO_4). These two reagents gave satisfactory results for acid soils but when it came to calcareous soils we find that the correlation between the amount of phosphorus extracted and plant response was rather poor. This was because the $CaCO_3$ content of these soils reacts with the acids, rendering them uneffective in extracting phosphorus. After further research on calcareous soils, carbon dioxide and later sodium bicarbonate were found to give good correlation between amount of phosphorus extracted and plant response.

The $CaCO_3$ content of calcareous soils not only caused troubles in phosphorus research but was also found to cause difficulty in iron nutrition. Lime induced chlorosis has been recognized for many years on these calcareous soils where iron is inactivated by complex combination of several reactions in either the soil or inside the plant or both. The pH and phosphorus content of the soil seem to have a major role in developing iron chlorosis. In first trials to cure iron chlorosis on calcareous soils by using the first chelating agent used in agriculture, ethylene diamine tetraacetic acid, (EDTA), no success was achieved because either the iron or the EDTA or both are inactivated. Later other chelating agents were produced to be used specifically on calcareous soils with satisfactory success.

Shortly after the first chelates were used, some evidence arose that the chelates might have an effect on phosphorus nutrition by increasing its availability (Bear, 1955; Brown et al., 1960; DeRemer, 1961). It would seem that a work covering this point could be profitable. The major objectives of this work were:

1. To study the effects of hydrogen and iron chelate added to the soil on phosphorus mobility, solubility, and availability to plant from different phosphorus sources.
2. To study the level of chelate concentration in regards to objective 1.
3. To study the phosphorus level in regards to objective 1.

Some minor objectives were:

1. To get more information on the function of chelates.
2. To increase our knowledge on translocation of nutrients within plants.
3. To study nutrient interaction within plants.

LITERATURE REVIEW

How the Chelates Function

Chelates have been used in agriculture for the past decade as a corrective for certain malnutrition deficiencies developed by plants (Stewart and Leonard, 1952). The answer to the question as to how chelates function is not yet satisfactorily answered. Wallace (1956) assumed that the soluble iron chelate is absorbed by the root and both iron and chelate move to the leaves. Once in the leaves the iron must be removed from the chelate before it will function in the metabolism of the leaf. How the iron is removed from the chelates in the leaf is unknown. Wallace (1956) thinks that sunlight may be the key since iron chelate solutions readily decompose in sunlight, and the reaction in leaves may be similar. He even goes further in emphasizing the adsorption of the complexing agent by the roots when he states that chelating agents without metals can enter plants and reactivate iron previously precipitated in the plant. In some of his other work, Wallace (1955) gives further evidence that the entire molecule of Fe-EDDHA is absorbed by plants. He based this conclusion after detecting the red dye of the Fe-EDDHA one day after applying the compound to the root media. The chelate is red only when complexed with iron.

The other viewpoint is suggested by Tiffin et al. (1960). They analyzed the exudate of zinnia, sunflower, and soybean plants growing in media containing EDDHA or ferric chelate of this acid, Fe-EDDHA, for total iron, chelated iron, and chelating capacity. The results showed that the average ratio of chelated Fe to total Fe was 1:12. In the same work iron concentration progressively decreased from the nutrient solution with a concomitant increase of iron in the plant and an increase in the chelating capacity of the nutrient solution. A conclusion was drawn that the plants selectively absorbed Fe and the EDDHA complex remained, for the most part, in the nutrient medium. In other work Tiffin et al. (1960) concluded that the primary role of iron chelates in plant nutrition appears to be that of making iron available to the root and very little emphasis can be placed on the absorbed chelating agent as an effective motivator of iron within plants. Brown et al. (1960) reported a reciprocal relationship between the concentration of the chelating agent in the root media and iron concentration in the top of red kidney beans. The concentration of chelating agent was found to be a factor affecting the capacity of roots to absorb iron. From this work, which was not designed to study the absorption of chelate components, we can reasonably conclude that if the plant could absorb the iron chelated molecule it would not develop iron chlorosis. But what could be understood here is that the iron molecule alone is absorbed leaving the complexing agent in the outside media.

Wallace (1960) complicates the picture further when he states that more chelating agent than metal was found in some plants supplied by metal chelate. He refers to some unpublished studies using Fe^{59}- labeled and C^{14}-labeled EDDHA in which he found equimolar amounts of the chelate and iron in the leaves, but he does not present a clear idea about the fate of the chelating agent, either absorbed from the growing media as such, or after separation from the metal. He does state,

> There is little evidence that synthetic chelating agents are metabolized in plants. Although iron chelating agents undergo oxidation in the presence of sunlight, and leaves are exposed to sunlight, there is no direct evidence that this oxidation is the manner in which chelating agents decompose in plants.

Hill-Cottingham (1957), using a spectrophotometric method to measure Fe-EDTA in the nutrient solution at intervals, found that a decrease in the concentration of chelated iron took place without the formation of any free EDTA, hence the conclusion that plants absorb the whole molecule. To explain how the iron is removed from the complexing agent he suggests that in ultra-violet light ferric EDTA is reduced to the less stable ferrous form and that repeated photoreduction and re-oxidation releases sufficient OH ions to precipitate ferric hydroxide. Under conditions which inhibit photoreduction, using red cellophane frames to cover tomato plants, it has been found that prevention of photoreduction of Fe-EDTA did not prevent its utilization by plants. The mechanism by which iron is released from the chelate is still without experimental evidence.

The writer has the feeling that the opinion of Tiffin et al. (1960) is more logical in explaining the function of chelates because it gives a more clear and comprehensive picture to what could be going on. If the chelating agents are left in the root medium, it is logical to assume that they would do something, and this something was first pointed out by Bear (1955) when he states that "there is some evidence that certain chelating compounds may have value in maintaining fertilizer phosphorus in a more highly available state in the soil." The work of DeRemer (1959) was the first designed to check the validity of Bear's statement. Using iron or aluminum phosphate as insoluble phosphorus sources he suggested a cycle in the growth medium which starts when the metal chelate gets in touch with roots and the plants remove the cation from the chelate molecule. If there is ferric phosphate present, the complexing agent can chelate some iron, leaving the phosphate in a soluble form, already available to the plants.

It is very interesting to probe in some work, even though not primarily designed to detect the chelate-phosphorus effects, and observe the relation between the chelate concentration in the medium and phosphorus concentration in the plant. The work of Brown et al. (1960) represents an excellent example. The phosphorus concentration increased in the plant from 0.29×10^4 ppm by using 0.16 M EDDHA in the nutrient solution to 0.35×10^4 ppm by increasing the EDDHA to 6 M EDTA, reported in the same work, increased the phosphorus concentration from 0.22×10^4 ppm to 0.42×10^4 ppm when the previously mentioned chelate concentrations were used.

Phosphorus Nutrition

Superphosphate and rock phosphate are common phosphorus fertilizers. From the field trials and laboratory experiments, the evidences are extensive indicating the superiority of the first over the second source. Ensminger (1950) states that superphosphate was a more effective source of phosphorus than raw phosphates. Ensminger and Pearson (1955) state that a combination of superphosphate and rock phosphate as a phosphorus fertilizer might be economical in some cases; however, this was not the case in the work they reported. Moschler et al. (1956) report that the availability of residual phosphorus from superphosphate, measured in terms of "A" value, was approximately four times that in rock phosphate. Rogers et al. (1953), in an extensive review of phosphate fertilizers, reported that experience has shown that the response of crops to rock phosphates has been inconsistent, whereas response to superphosphate on phosphorus defficient soils has been consistent. Also they reported some factors to influence the response to rock phosphate beside the initial phosphorus level in the soil. Among these factors are the fluorine content, fineness, soil reaction, feeding power of the plants, organic matter content of the soil, and rate of application.

The problem of phosphorus supply to the plant is not, however, solved by the addition of soluble phosphorus sources to the soil because there are many factors that render the applied soluble phosphorus to less soluble forms and, consequently, less available to the plant. Olsen (1953), in an excellent review on the subject, mentioned the following items as the limiting factors to the reaction between the applied phosphorus and the soil:

1. Soil physical properties
2. Reaction with clay: where it had been shown that a calcium saturated clay absorbs more phosphate than a sodium-saturated clay.
3. Effect of calcium carbonate: which depresses phosphorus absorption by both decreasing solubility of soil phosphate and the tendency of $CaCO_3$ to maintain a high pH. An absorption of phosphate onto the surface of the $CaCO_3$ particles is quite possible.
4. pH of the soil: for a calcareous soil the results are in general agreement that phosphate solubility is at minimum in the range of pH 7.0 to 7.5 and increases with either an increase or decrease in pH from this range.
5. Salts on root membrane: with a soil of a pH value of 6.96 it was found that the chloride and nitrate of Na, K, Mg, and Ca increased the total phosphorus content of pea plants. These results were explained by the fact that salt content affects the phosphorus uptake by plants through two factors (1) the effect on phosphate solubility in the soils, and (2) the effect

of the salt on the root membrane.

6. Soluble salts in the soil: classically salts that do not have a common ion with the slightly soluble calcium phosphate increase its solubility by decreasing the ions activity (if Ksp, which is the product of the ions concentrations times their activity, has to be constant). Salts that have common ions with calcium phosphate decrease its solubility through the common effect action, although this effect is counteracted to some extent by decreasing the ions activity. In soil systems, however, many contradictory results have been reported, where NaCl, KCl, K_2SO_4 all decreased the solubility of phosphate in calcareous soils. These results were explained by the effects of the added salts on increasing the solubility of $CaCO_3$ which provides more Ca^{++}, which in turn depresses the solubility of calcium phosphate. Some other work showed that the effect of cations in depressing phosphate solubility to have the order Ca > Mg > K > Na, and the effects are less marked with sulfate than with chloride and nitrate.

The fixation of the applied phosphorus in soils brings up another problem beside that of decreasing the solubility, that is, the uneven phosphorus distribution throughout the root zone in that there is a piling up of the phosphorus in the surface. Tisdale and Nielson (1958) mentioned that the studies conducted in Iowa have shown that phosphorus plowed down in the fall was more effective on corn than phosphorus disked in the spring. The advantage of the plow-down application was thought to be related to deeper replacement and less mixing with the soil. Deep fertilizer applications were tried to get the phosphorus down, to reach lower in the soil where most of the active roots exist or where the available moisture is relatively higher than the surface. Stanberry (1948) found that the maximum phosphorus uptake from applied fertilizer was greatest when the fertilizers were applied in bands 2 to 4 inches deeper than the seed. There was little difference between the two depths when the soil was kept moist, but considerably more phosphorus was obtained from the 4-inch depth under drier conditions. Phosphate placed in bands either 2 or 4 inches deeper than the seed was more efficient than the broadcast application. Haddock (1957) found that sugar beets used the phosphorus fertilizer more efficiently early in the season when it was banded 6 inches deep than when it was banded 3 inches deep or broadcast. In the same reference it was reported that methods of application and position of the fertilizer are important in relation to the type and development of the root system and product of the fertilizer reaction with the soil. Although phosphorus movement appears limited in most soils, water soluble fertilizers may move either in solution or as discrete particles. This is in accordance with the results of Thor (1933) who found that the downward movement of phosphorus following an application of phosphate rock was greater in open textured soils. He found an appreciable amount of phosphorus moved to the 24-inch depth in open textured soils. He believes that phosphate rock was carried down by gravitational water and that the movement was largely mechanical.

Anyway, this movement introduces adequate distribution of fine particles in the feeding zone where roots may maintain contact with a large phosphate surface. In other words, the phosphate may become positionally available.

The literature outlined above refers to the broad field of the problem in which the current work has interest. More references will be referred to in discussing some of the details of the results. Since the writer has been unable to find any similar work the results might serve for initiating and developing a new knowledge. In other words, this pioneer work might represent a new phase in phosphorus-iron-chelate-soil-plant relations.

PLANT GROWTH EXPERIMENT

Methods and Procedure

Moscow tomato seeds were germinated in a sand medium, June 27, 1961. Transplantation took place on July 13, 1961, into one-gallon glazed crock containers which had drain holes in the bottom. Four plants were transplanted into each pot. Fine sand was used as a root medium. To investigate the effect of chelates on phosphorus availability two sources of phosphorus were chosen, treble superphosphate as a soluble source and rock phosphate as an insoluble source. A modified Hoagland solution No. 1, full strength (1950), was used. By this modification the solution contained no phosphorus or iron. See table 1.

From rock phosphate, two phosphorus levels were used, 66 and 132 pounds phosphorus/acre (150 and 300 pounds P_2O_5/acre), while only one level of superphosphate, 66 pounds phosphorus/acre, was used. The fertilizers were broadcast on the surface of the sand and a slight mixing into the subsurface was done; the phosphorus application was made just before transplanting.

Iron was supplied either as iron chelate or as iron citrate, according to the different treatments. In both cases the concentration was 1.14 ppm iron.

EDDHA (ethyline diamine di-o-hydroxyphenylacetic acid) was used as the chelate material, either in the acidic form, H-EDDHA, or in iron chelated form, Fe-EDDHA. In the case of the H-EDDHA the concentration

Table 1. Composition of the nutrient solution

Major element stock solution			
Stock solution number	Compound in stock solution	Stock solution in the nutrient solution	Ion concentration in the nutrient solution
		ml/liter	ppm
1	M. KNO_3	6	NO_3 : 868
2	M. $Ca(NO_3)_2$	4	K : 234
3	M. $MgSO_4$	2	Mg : 48.6 S : 192
4	0.0466 M. H_3BO_4	0.5	B : 2.5

Minor element stock solution[a]		
Compound	Compound in stock solution	Ion concentration in the nutrient solution
	g/liter	ppm
$MnCl_2 4H_2O$	1.81	Mn : 0.5
$ZnSO_4 7H_2O$	0.22	Zn : 0.05
$CuSO_4 5H_2O$	0.08	Cu : 0.02
$H_2MoO_4 H_2O$	0.02	Mo : 0.01

[a] A supplementary stock solution which will supply manganese, zinc, copper, and molybdenum. One ml of this solution is added to one liter of the major nutrient solution.

of the chelating agent was used equivalent to that supplied by chelating agent in Fe-EDDHA which would supply iron concentration of 1.14 ppm. Chelate applications were 3 days after transplanting. The reason for choosing EDDHA in this work was its several advantages as mentioned by Wallace et al. (1955) which could be summarized as follows: (1) greater stability, (2) relatively less toxicity, and (3) absence of appreciable fixation on the clay fraction of the soil.

To sum up the experiment, the treatments could be summarized as follows:

1. $R_1 + H_2O$
2. R_1 + Fe - Chelate
3. R_1 + H - Chelate
4. $R_2 + H_2O$
5. R_2 + Fe - Chelate
6. R_2 + H - Chelate
7. $S + H_2O$
8. S + Fe - Chelate
9. S + H - Chelate

Where R_1 and S represent the rock phosphate and treble superphosphate supplying 66 pounds phosphorus per acre, and R_2 represents an amount of rock phosphate that supplies 132 pounds phosphorus per acre. Each treatment consisted of four replications and each replication consisted of four plants. The modified Hoagland's solution was added daily in sufficient quantity to allow for drainage from the holes in the bottom of the pots. The pots were distributed in the greenhouse in a completely randomized design. Iron was added in periods which ranged from 4 to 6 days. Iron as iron citrate was added a day before the hydrogen chelate to eliminate initial intimate contact between the iron and the chelating agent.

Most of the plants did very well except on 5 pots, where due to some drainage trouble, the plants were far less vigorous than their partners within the same treatment. The plants were later eliminated from the yield account and chemical analysis.

On August 14, 1961, extensive observations were taken on the plants. The different nutrient deficiency symptoms were noted and the relative vigor of growth was reported. The heights of the four plants in each pot were averaged to give a number representing the height of the replication, and flower initiation was reported. On the next day the flowers were removed and all blossoms kept off until the harvest. On August 27, 1961, the plants were not irrigated to get ready for harvest. The height of the plants was recorded. On August 28, 1961, the harvest was conducted in such a way as to obtain the root system of the plants. Tap water was directed into the pot to remove most of the sand and the plants were pulled out of the pot in a smooth way. The plants of each pot were put in separate sacks and transferred to the laboratory, where the plants were again washed by tap water and then by distilled water. Roots were separated from the aerial parts, each put into a separate sack, and dried in a forced air drier at 75 F for 36 hours. The aerial parts were weighed. It was not possible to weigh the roots because some sand particles still adhered to them. Two sieves were used to get rid of this sand. The upper sieve (holes 1 mm radius) was coarse enough to pass sand particles and the fine root parts, but retain the coarse roots. The second sieve (40 mesh) was fine enough to pass the fine roots but retain the sand particles. After sieving, the coarse and fine roots were added together and weighed.

After grinding the plant materials, samples were taken and digested in a mixture of HNO_3 and $HClO_4$ as described in USDA Handbook 60 (1954). Phosphorus was determined by the molybdate-metavanadate method. Calcium was determined by the versenate method and potassium was determined by the flame-photometer. Iron was determined by the orthophenanthroline method.

Results

Growth and development

Qualitative measurements

Color and size.--On August 14, 1961, an extensive check was made on the plants in each treatment. Some plants were missing because of incomplete drainage. Others were very slightly yellow due to a suspected nitrogen deficiency. These plants were only in the three treatments involving superphosphate as the phosphorus source. The plants were large and well developed. The nutrient supply to all plants was increased and the slight yellowing disappeared.

Flower initiation.--Glover (1953) found that corn reached the stages of tasseling and silking at an earlier date when the phosphorus supply was ample. In the work reported here, there was no correlation between the added phosphorus or the availability of the phosphorus supply at flowering. Wall (1938) found that the maturity date for plants was advanced little or none by phosphorus fertilizer.

It is, however, interesting to note the correlation between chelate treatment and early flowers. In those pots where no chelate was applied, there were flowers on 4 out of the 11 pots on August 14. Out of the 20 pots receiving chelate, 11 showed flowers, 6 under the iron chelate and 5 under the hydrogen chelate. This work was not set up to study this phase of the observations and no more can be assumed. It does introduce a question and more investigations are invited.

Quantitative measurements

To make the statistical analyses, the average of each treatment was inserted in the place of the missing data for those treatments where the plants did not survive. This procedure was followed in all the analyses that were evaluated.

Heights on August 14.--From table 2 we see that neither the phosphorus source or level that was given nor the chelate treatments had any effect on plant height at this time. In the statistical analysis for this measurement, although phosphorus or chelate have no significant effect, the interaction is significant. This situation is solved by Cochran and Cox (1960) when they state, "If two factors are not significant but their interaction was significant, it is advisable not to lay much stress on the interaction in the absence of other conformatory evidence." Such evidence is not available.

Final heights.--The same conclusion that was given for the heights on August 14 could be drawn concerning the final height. These conclusions are supported statistically. If the heights on August 14 are subtracted from the final heights to give the rate of development between two dates, it will be noticed that in all treatments an increase that runs around 100 percent was achieved. A difference in height affected by treatments is hardly expected if it is known that the height of the living being is well controlled by hereditary genes which are not very sensitive to the outside environment.

Roots yield.--If root yield is regarded, it is clear that there is no effect of phosphorus treatment on their growth. This disapproves the

Table 2. Growth and yield of tomato plants subjected to the different treatments[a]

Treatment[b]	Height		Dry weight		
	August 14	Final	Roots	Tops	Total
	inches			grams	
$R_1 + H_2O$	11.5	22.7	6.2	32.3	38.4
R_1 + Fe-EDDHA	10.0	20.2	6.9	33.0	39.9
R_1 + H-EDDHA	14.0	22.7	7.8	36.8	44.6
$R_2 + H_2O$	13.0	20.4	8.9	36.1	45.0
R_2 + Fe-EDDHA	11.5	21.4	6.8	31.5	38.3
R_2 + H-EDDHA	12.7	21.5	7.0	35.4	42.4
$S + H_2O$	11.5	20.9	7.1	37.2	44.3
S + Fe-EDDHA	12.7	21.9	7.6	39.3	46.9
S + H-EDDHA	11.5	20.5	7.3	36.5	43.8

[a] Average of four replications.

[b] R_1 = 66 pounds phosphorus supplied from rock phosphate; R_2 = 132 pounds phosphorus supplied from rock phosphate; S = 66 pounds phosphorus supplied from treble superphosphate.

once-held impression about the stimulating effect of phosphorus on root growth, but supports the conclusion given by Black (1957) where he states that

> if roots refers to the absorbing roots, phosphorus does not seem to have any special stimulating effect. In fact, treatment of phosphorus deficient plants with P fertilizer ordinarily increases the yield of the above-ground parts to a greater extent than the absorbing roots. The explanation given is that plants deficient in phosphorus tend to be high in carbohydrate. When conditions are made more favorable for utilization of the carbohydrates in growth, as when the supply of P is increased, the proportion of the carbohydrate translocated to the root decreases.

With respect to the chelate treatments, it can be concluded that it had no critical consequences on root growth.

Top yield.--The growth of tops as affected by treatment reveals several trends. There has been a significant gain in growth by using superphosphate as a phosphorus fertilizer, while there has been little response to doubling phosphorus level from rock phosphate. These results add to the numerous citations reported by Rogers et al. (1953), which confirmed the superiority of superphosphate over rock phosphate in greenhouse experiments.

Chelate treatment did not develop significant response, although it can be seen that iron chelate has decreased the yield while hydrogen chelate has increased it, relative to the non-chelate treatment. This agrees with DeRemer's (1959) findings.

Total yield.--The effect of the different phosphorus and chelate treatments on the total yield of tomato plants follows the same pattern of their effect on top yield. Only the significant response is lost here because the effect on root growth was too faint to be distinguished statistically.

Chemical analyses

After the plants were harvested they were separated into roots and tops. Following drying and grinding the plant material was analyzed for phosphorus, iron, calcium, and potassium. The results are reported in tables 3 and 4.

Chemical analysis of roots

Phosphorus.--As can be seen from table 3, phosphorus source and level, as well as chelate treatment, gave different phosphorus content. The rate of increase was 65 and 35 percent for superphosphate and the high level of rock phosphate, respectively, beyond that of the low rock phosphate level. These results support many works that have been designed to prove the superiority of superphosphate. Fried and MacKenzie (1949) reported this superiority under moderately neutral soils. Chin et al. (1959) reported the same results. Among the same phosphorus source and level the chelate treatment increased phosphorus content sometimes up to 35 percent above that of the control.

Iron.--None of the treatments used, either with respect to fertilizer phosphorus or chelate, had a statistically different effect on iron content in roots. However, several trends could be pointed out. Iron content decreased as phosphorus supplied by the fertilizer increased. Chelate treatment increased iron uptake over that of the control. Hydrogen chelate increased iron uptake over that obtained by using iron chelate. This result might seem strange but it supports some of DeRemer's (1959) results.

Calcium.--No treatment produced a significant effect on calcium content of the roots. With respect to phosphorus source and level, such an effect would not be expected because of the fact that rock phosphate is as good a calcium source as superphosphate (Thurlow et al., 1960, and

Table 3. Nutrient concentration in roots of tomato plants[a]

Treatment[b]	P	Fe	Ca	K
	microgram/gram		percent	
$R_1 + H_2O$	220	167	3.20	3.92
R_1 + Fe-EDDHA	274	208	2.84	3.86
R_1 + H-EDDHA	279	237	2.98	3.69
$R_2 + H_2O$	286	182	2.91	3.70
R_2 + Fe-EDDHA	372	191	3.23	4.17
R_2 + H-EDDHA	383	224	3.95	2.94
$S + H_2O$	423	125	3.88	2.60
S + Fe-EDDHA	466	161	3.01	2.96
S + H-EDDHA	398	194	2.66	3.05
L.S.D. for leaching solution (.05)	26	n.s.	n.s.	n.s.
L.S.D. for phosphorus (.05)	14	n.s.	n.s.	0.39

[a] Average of four replications.

[b] See footnote b of table 2, page 19.

Table 4. Nutrient concentration in top of tomato plants[a]

Treatments[b]	P	Fe	Ca	K
	microgram/gram		percent	
R_1 + H_2O	140	45	3.68	6.03
R_1 + Fe-EDDHA	140	28	3.48	5.99
R_1 + H-EDDHA	150	55	3.24	5.99
R_2 + H_2O	135	33	2.85	5.85
R_2 + Fe-EDDHA	155	41	2.73	6.34
R_2 + H-EDDHA	148	71	2.84	6.09
S + H_2O	250	73	2.47	6.25
S + Fe-EDDHA	213	77	2.27	6.41
S + H-EDDHA	240	94	2.61	6.55
L.S.D. for leaching solution (.05)	n.s.	n.s.	n.s.	n.s.
L.S.D. for phosphorus (.05)	11	11	0.10	0.18

[a] Average of four replications.

[b] See footnote b of table 2, page 19.

Fried et al., 1949). Chelate treatments, too, were not expected to give different calcium analyses, even though some of calcium would be complexed by hydrogen chelate. The fact that calcium chelates have small stability constants means that soon after their formation the calcium would be substituted for by a cation that forms a stronger chelate. Also, the calcium supply was in excess to that which might be required for forming calcium chelate and plant requirement, because it was supplied by the fertilizer as well as by the nutrient solution.

Potassium.--Potassium contents of the roots were significantly affected by phosphorus supply in that they decreased as the phosphorus supply increased. This could be attributed to either uptake antagonism, which is difficult to visualize, or to some factors that stimulated potassium movement to the aeraial parts. Chelate treatment was not expected to give significantly different results. The same reasons mentioned with respect to calcium hold here also.

Chemical analysis of tops

Table 4 reports the analysis of the investigated nutrients in the tops of plants.

Phosphorus.--Within rock phosphate treatments, doubling the level of the applied phosphorus did not affect the level of phosphorus in the tops, but superphosphate treatments greatly increased the nutrient content beyond that obtained by either level of rock phosphate.

The element content as affected by chelate treatments was negligible. This might be due to some factors that depressed the advantage of chelate found in increasing the content in roots.

Iron.--Iron content increased with increasing phosphorus level. Although this result disagrees with many reports, it agrees to some extent with Miller et al. (1960) who found that iron concentration in the leaves was not affected by phosphorus concentration in the growth medium. This result could be due to iron precipitation in the tops as iron phosphate (Biddulph, 1951). Chelate treatments had no significant effect on iron content but the consistant trend of the content pattern supports the discussion cited previously about iron content in roots as affected by chelate.

Calcium.--Calcium content in tops has decreased by increasing phosphorus level. The lower content could be attributed to phosphorus iron-calcium complex formation and precipitation in roots. Biddulph (1951) states that under neutral growth medium, calcium is an important constituent of phosphate precipitates. Chelate treatment has decreased the calcium content below that of the non-chelate treated plants, but the effect is not significant.

Potassium.--The content of potassium increased in the same direction where phosphorus and iron decreased. This supports Perkins and Purvis' (1954) findings. Brown (1955) found that the increase of phosphorus in the sap of soybeans was associated with high potassium concentration.

Discussion

Nutrient content

If growth is regarded, chelate treatment did not develop significant response, although it can be seen that iron chelate has decreased the yield while hydrogen chelate has increased it, relatively to the non-chelate treatment. This agrees with DeRemer's (1959) findings. DeRemer explained his findings as a result of the small iron supply by hydrogen chelate compared to iron chelate which would allow the plant to utilize the phosphorus it received. In this work, as revealed by the chemical analysis, hydrogen chelate treatment contained more iron than iron chelate treatments. So, if precipitation of phosphorus in roots is the reason for depressing the yield, iron uptake, in situ, is not important, but the stress should be applied to how the iron is supplied in each case. Brown et al. (1959, 1960, 1961) reported that there is a competition between roots and the chelating agents for possessing iron molecule. This basis could serve in explaining the high iron concentration obtained by using hydrogen chelate rather than iron chelate. The chelating agent is assumed to act just as a carrier of iron from the medium to the root, but is not absorbed by the roots. In the case of iron chelate, the root has to expend a relatively greater effort to extract iron molecule from the chelate structure. In the case of hydrogen chelate, the same magnitude of effort is not required because upon addition of hydrogen chelate to the root medium, it comes in contact with an abundant supply of cations, such as Ca, Mg, Zn, Cu, K, and Mn that can be chelated by the acid chelate, and this would decrease the ability of the chelating agent to chelate all the iron supply in the medium. In other words, the chelating ability of the acid chelate in this case would be shared by many cations leaving more iron in the ionic form in the soil solution. If this assumption is correct, it leads to the conclusion that in the acid chelate treatment, plants had two sources of iron, iron supplied in the ionic form and that amount that had been chelated to form iron chelate.

There is another possibility which explains the relatively low iron content of the iron chelate treatment extracted from information cited by Martell and Calvin (1952). They state that by increasing the number of the donor groups in the chelate, the stability constant of the chelate increases. So it could be visualized that upon iron chelate application to the medium, where phosphate is available, phosphate would be incorporated in the chelate structure as an active group forming mixed chelate, which would be expected to have greater stability constant than the original chelate, consequently iron availability to the plant would decrease. Chelate has increased phosphorus content in roots. There are several possibilities to explain why this was so. First, the chelate has a significant role in increasing phosphorus solubility on its own, or the plant is involved in the mechanism. Second, the chelate increased iron uptake and this iron was precipitated in roots as iron phosphate. Third, the chelate, though encouraging by some means the phosphorus uptake, had in some way depressed the movement of phosphorus to the aerial parts. In tops, if phosphorus content of rock phosphate treatments is related to the superphosphate treatment, it would seem that the efficiency of the higher level of rock phosphate was greater than the lower level if roots were regarded, but the two levels have the same efficiency if the tops were under consideration. Inactivation of phosphorus in roots by forming iron phosphate is eliminated as a reason of this decreased efficiency because iron content in roots with the lower level of rock was larger than that of the higher level while the content was reversed in the tops. Inactivation of phosphorus as calcium phosphate in the roots is a possible explanation. This is supported by the fact that calcium concentration in tops of this treatment was relatively low.

To have a more comprehensive idea about the effect of the different treatments on phosphorus and iron uptake, nutrients content have been related to each other in several combinations. The following section is devoted to this purpose.

Nutrient translocation ratios

Translocation has been used in plant studies to indicate how much of the applied element has moved from the point of application to another point. Time may or may not be a factor under consideration. In this work the term translocation will be used to indicate a distribution concept; in other words, how much of the absorbed element is located in roots and how much in the tops. The ratio of these two amounts facilitates the contrast. Table 5 gives the ratios of the four nutrient elements analyzed.

Phorphorus

Iron chelate treatment depressed the phosphorus movement from the roots to the tops more than any other treatment. One reason for that might be the formation of the immobile iron phosphate. From the first sight, suspicion could be thrown on the validity of this reasoning because the analysis revealed more iron uptake with hydrogen chelate treatment than with iron chelate, but this fact could not be serious if we discuss the way by which iron is supplied in each case. With iron chelate as an iron source, the supply is given one at a time which makes it more effective in precipitating phosphorus. Hydrogen chelate supplied the plants with more iron, but the supply was in a gradual pattern. If we review the method under which the hydrogen chelate treatment was imposed, we find that iron citrate was added in the first day, so the plant will absorb some iron, and the next day the hydrogen chelate was added which will complex some of the remaining iron and consequently provide more available iron.

Table 5. Nutrient translocation ratios for tomato plants[a]

Treatment[b]	P	Fe	Ca	K
$R_1 + H_2O$	1.57	3.75	0.86	0.65
R_1 + Fe-EDDHA	1.95	7.42	0.81	0.64
R_1 + H-EDDHA	1.86	4.30	0.91	0.61
$R_2 + H_2O$	2.11	5.51	1.02	0.63
R_2 + Fe-EDDHA	2.40	4.65	1.18	0.65
R_2 + H-EDDHA	2.40	3.15	1.39	0.48
$S + H_2O$	1.69	1.71	1.57	0.41
S + Fe-EDDHA	2.18	2.09	1.32	0.46
S + H-EDDHA	1.65	2.06	1.01	0.46

[a] Expressed as the ratio of concentration in roots:concentration in tops.
[b] See footnote b of table 2, page 19.

The high phosphorus content of roots does not necessarily, in all cases, indicate phosphorus precipitation. Biddulph (1952) indicates that by increasing phosphorus level in the solution culture, the rate of increase in phosphorus content in the roots was higher than in the stems and leaves. With still higher phosphorus concentration in the solution, tissue concentrations began to level off. The results of table 5 support Biddulph's observation, where we find that the ratio has increased by increasing phosphorus level from the low to the high level of rock phosphate, then decreased by increasing the phosphorus supply by superphosphate.

Iron

Phosphorus level has a prominent effect on iron translocation ratio. The translocation was increased by an increase in phosphorus level. This sounds like a contradiction to many reports (Neher, 1958, and Doney, 1959). But it could be said that conditions which inaugurate iron precipitation and decrease the available iron supply might also develop some kind of "thirst" within the plant for iron which might improve iron translocation relative to that observed when iron nutrition is under "no tension." This concept of "iron tension" has some support in work by Rediske and Biddulph (1953) and Doney (1959) where they found that the best translocation of foliar applied iron occurred when the plants were grown in a solution containing no iron. Superphosphate treatments gave the best translocation ratio. Beside the postulation of "iron tension" that improved translocation, the effect of superphosphate on the growth of tops might have stimulated some physical processes which increased the iron content in the tops.

Non-chelate treatment gave better translocation than hydrogen chelate treatment because the latter treatment supplied more iron, also more phosphorus. The hydrogen chelate would be expected to produce more iron phosphate, consequently less iron translocation.

Calcium

The effect of different treatments on calcium translocation is not as pronounced as it was in the case of iron; also the pattern is not as easily distinguished. However, it can be seen that the ratio of calcium translocation is in the reverse direction of iron translocation. In almost all cases, whenever the ratio of iron translocation was at a minimum, calcium ratio was at a maximum. Biddulph (1951) reported that under conditions of neutral pH's phosphorus precipitate in the plant is a complex of phosphorus, iron, and calcium. The data reported here suggest that phosphorus-iron compounds have the priority, and the excess of phosphorus after iron phosphate precipitation reacts with calcium to form insoluble compounds of calcium phosphate, which might accumulate on the previously formed iron phosphate giving a final complex of phosphorus-iron-calcium compound.

Potassium

The level of rock phosphate did not have much effect on the potassium translocation ratio. However, phosphorus from superphosphate influenced the ratio. This is to say that under conditions of phosphorus abundance, not necessarily leading to better growth as indicated by a greater yield, the physiological processes in the plants were active in such a way as to acquire and accumulate greater amounts of potassium. Smith (1955) has shown potassium absorption by plants to be in an inverse proportion to calcium concentration in the medium. The same kind of relation could be figured out concerning the ratio of potassium and calcium translocation. The ratio of iron translocation agrees to some extent with the direction of potassium translocation. Millar (1959) states that potassium improves iron translocation within the plant.

Nutrient interrelation

The concentration of the elements analyzed has been related to each other in different combination to show some of the possible interactions between them.

In roots

Phosphorus to iron ratio.--Table 6 shows nutrient interrelation in roots. The phosphorus:iron ratio (P/Fe) gives a practical evaluation to the actual effect of chelates on increasing consequences which limit the ability of the plant to use the extra phosphorus that has been rendered available.

The ratio was at a minimum and a maximum under the low level of rock phosphate and superphosphate treatments, respectively. With the lowest ratio, the plant did not develop phosphorus deficiency symptoms, maybe because it is still far from the critical ratio, or also because of the extreme mobility of phosphorus within the plant (Biddulph, 1952). The high ratio was not associated by iron chlorosis symptoms, as suggested be DeKock et al. (1955). There might be two reasons why this is so. First, the ratio did not reach the chlorosis critical level, and second, the tomato variety used in this work might be resistant to this problem. Information proving or disproving this resistance is not available.

DeRemer (1959) found that chelates decreased the P/Fe ratio. This was under some conditions of the present study but the overall effect contains a possible interaction between the amount of phosphorus and

Table 6. Nutrient interrelations in roots of tomato plants[a]

Treatments[b]	P/Fe	Ca/P	Ca/Fe	Ca + K/P	Ca + K/Fe
R_1 + H_2O	1.31	145	191	323	426
R_1 + Fe-EDDHA	1.31	104	136	264	348
R_1 + H-EDDHA	1.18	107	125	239	281
R_2 + H_2O	1.57	102	159	231	363
R_2 + Fe-EDDHA	1.94	87	169	198	387
R_2 + H-EDDHA	1.70	103	176	179	307
S + H_2O	3.38	92	310	153	518
S + Fe-EDDHA	2.89	65	186	129	370
S + H-EDDHA	2.05	67	137	143	294

[a] Expressed as concentration ratios.
[b] See footnote b of table 2, page 19.

iron supplied, which complicates the picture. Under conditions of limited phosphorus supply, such as the lower level of rock phosphate, chelate had produced a significant effect on increasing phosphorus availability to the plant, but in the same time it provided more iron. It is a matter of relativity then, on how far the availability of both phosphorus and iron would be increased, that affects the ratio. For example, under this level of rock phosphate, iron chelate increases phosphorus availability. If we assume that the effect of the two chelate forms on increasing phosphorus availability is about the same, which is supported by the equal phosphorus content in both cases, the prime factor that affects the ratio would be iron. It was explained previously why hydrogen chelate treatment rendered more iron to the plants and thereby decreased the ratio.

Under the higher level of rock phosphate, although chelate increased phosphorus uptake far beyond the non-chelate treatment, the ratio does not reflect this increase because of the concomitant change in iron uptake. The efficiency of iron chelate was apparently greater in the direction of increasing phosphorus uptake rather than iron uptake. Hydrogen chelate increased the phosphorus uptake to the same extent as did the iron chelate but the P/Fe ratio is less than for the iron chelate treatment because of the increased iron uptake.

Calcium to phosphorus ratio.--Generally, the highest and lowest calcium:phosphorus (Ca/P) ratios were associated with the non-chelate and chelate treatment, respectively. The high ratio could be a result of either high calcium content or low phosphorus content. The low phosphorus content of the non-chelate treatments has been shown and explained previously.

It has been mentioned before that calcium could be complexed by the chelate used. In the case of hydrogen chelate the reaction is a direct one between the chelating agent and the calcium ions in the soil solution. In the case of iron chelate, the chelating agent is released in a free form capable of chelating calcium when the iron is removed. In any event, calcium bounded to the chelate did not appear to have any significance in the calcium concentration of the roots, but when we combine the different parts of the picture, the chelate effect is clearly seen. Another point to be mentioned here is that the Ca/P ratio was high under conditions where iron content was not at its maximum. Biddulph (1951) mentioned that calcium is an important part in the phosphorus precipitates within the plant.

Calcium to iron ratio.--It seems that the calcium:iron (Ca/Fe) ratio was not very sensitive, at least in a direct manner, to the phosphorus levels as are the ratios discussed previously. However, it seems too that effect of chelate treatment on the ratio has some interaction with phosphorus level. In the low rock phosphate level the ratio is at its maximum and minimum under non-chelate treatment and hydrogen chelate treatment, respectively. This is due to two reasons: (1) the limited uptake of iron in the first treatment and (2) the high iron uptake and limited supply of calcium in the second treatment. The first and second point could be thought to have some kind of interaction where the limited supply of iron in the first non-chelate treatment presented a chance to calcium to be precipitated in roots as calcium phosphate. In the high level of rock phosphate the situation looks to be the reverse, but checking the nutrients content reveals the fact that there has been relatively little increase in iron content by chelate treatment. In the superphosphate treatment, nothing new could be added here except that the very high value of the ratio in the non-chelate treatment is probably due to the very low iron content in the roots, presumably due to iron precipitation outside the roots, which encourages calcium phosphate formation in the roots.

Calcium plus potasssium to phosphorus ratio.--The calcium plus potassium:phosphorus (Ca + K/P) ratio follows the Ca/P ratio. In both cases phosphorus level has its effect in decreasing the ratio by supplying more phosphorus. Within the same phosphorus treatment the ratio in the chelate treatment was less than in the non-chelate treatment, with hydrogen chelate treatment consistently the lower.

Calcium plus potassium to iron ratio.--The calcium plus potassium: iron (Ca + K/Fe) ratio has the same pattern of Ca/Fe ratio. Though less sensitive to phosphorus supply, there apparently has been the same interaction between the chelate effect and phosphorus level in the same way discussed in Ca/Fe ratio.

In the tops

Table 7 presents the data for the ratios of the various nutrient elements found in the plant tops.

Phosphorus to iron ratio.--For the most part, the iron concentration was responsible in fixing the value of the P/Fe ratio rather than the phosphorus concentration. In the lower level of rock phosphate there was a small change in phosphorus concentration, while iron fluctuated according to whether chelates were used or not. The iron content was very low in the tops of iron chelate treatment; presumably iron has been inactivated in roots through a reaction with phosphorus. This explanation is supported by checking the ratios of nutrient translocation where this particular treatment gave the highest P/Fe ratio

Table 7. Nutrient interrelations in tops of tomato plants[a]

Treatments[b]	P/Fe	Ca/P	Ca/Fe	Ca + K/P	Ca + K/Fe
$R_1 + H_2O$	3.11	262	817	650	2024
R_1 + Fe-EDDHA	5.00	248	1242	676	3382
R_1 + H-EDDHA	2.73	216	589	615	1678
$R_2 + H_2O$	4.09	211	863	644	2636
R_2 + Fe-EDDHA	3.78	176	665	585	2212
R_2 + H-EDDHA	2.09	191	400	603	1257
$S + H_2O$	3.42	98	338	348	1195
S + Fe-EDDHA	2.76	106	294	407	1127
S + H-EDDHA	2.55	108	277	381	974

[a] Expressed as concentration ratios.
[b] See footnote b of table 2, page 19.

(lowest iron translocation) in the whole experiment.

On the contrary, the efficiency of hydrogen chelate, under this level of rock phosphate, in maintaining iron to the tops of the plants has been superior to its efficiency in supplying phosphorus which caused a decrease in the ratio to an extent which surpassed even the non-chelate treatment. In the higher level of rock phosphate as well as the superphosphate treatments, the pattern of P/Fe ratio is the same, i.e., the highest ratios were obtained in the non-chelate treatment and the lowest in hydrogen chelate treatments. These results could be regarded as the effect of chelate on increasing phosphorus concentration while not increasing iron concentration to the same extent. In the overall experiment, the ratio is at maximum in the lower level of rock phosphate and at minimum in the superphosphate treatment. The reason for this could be hardly explained because it is expected that phosphorus, which is a major nutrient taken up and used by plants in greater quantities than iron, should have a more important role in fixing the value of the ratio. Since the power of superphosphate in supplying phosphorus is much greater than rock phosphate, it is expected that its treatment should have greater value, but this was not the case. Another possibility in explaining the low ratio in superphosphate treatment could be thought of as a result of the better growth which diluted phosphorus concentration (Seay and Weeks, 1955). However, if the extra growth of plants has diluted phosphorus concentration there is no justifiable reason to assume that this growth did not dilute the iron concentration.

Comparing the leaching solution on the basis of the whole experiment indicates that the highest value of the ratio was associated with iron chelate treatment. That is because its efficiency in supplying more phosphorus, while a part of the absorbed iron was inactivated in the roots.

Calcium to phosphorus ratio.--The ratio of calcium:phosphorus (Ca/P) was less in chelate treatments. This can be attributed to several possibilities. The first is the effect of chelate in maintaining a higher phosphorus content. The second is the possible effect of chelate in complexing some of the calcium, which has decreased its uptake or translocation to the tops. A third possibility is the inactivation of calcium in roots. In the overall experiment, the Ca/P ratio was decreased by increasing phosphorus level. This could indicate that calcium content in plant tops was not increased to the same extent that phosphorus was upon increasing phosphorus supply.

Calcium to iron ratio.--By increasing the phosphorus supply, the Ca/Fe ratio tended to decrease. This indicates that the phosphorus source was not a critical factor in calcium supply. Even if there was an effect, the calcium supplied in the nutrient solution would have been enough. The lowering of the ratio, then, by increasing the phosphorus supply is a result of the relative increase in iron content more than a decrease in the calcium content. There is another factor to be considered here; that is, the decrease in calcium content by increasing phosphorus supply. This had been explained previously as the priority of iron and calcium to form calcium-phosphorus precipitates. With adequate phosphorus supply calcium would represent a good share of phosphorus precipitates. If chelate treatments were regarded in the overall experiment, it can be seen that the ratio was least in the hydrogen chelate treatment. The major factor in this decrease is increasing iron content while complexing calcium stands as a minor factor.

Calcium plus potassium to phosphorus ratio.--The pattern of the Ca + K/P ratio resembles to a great extent the Ca/P ratio. Beeson et al. (1944) found that as calcium content of leaves increased the potassium content decreased. Smith (1955) found that potassium absorption was inversely proportional to calcium absorption. On such a basis the ratio of Ca + K/P is expected to be different from Ca/P, but it could be said that plant absorption of cations and anions has equalized the two ratios. Another possibility is provided by Millar (1958): "It is suggested that the equivalent cation content, particularly calcium, magnesium, and potassium of a given plant is approximately a constant."

Calcium plus potassium to iron ratio.--The conclusions mentioned in discussing Ca/Fe ratio could still hold for the Ca + K/Fe ratio.

LEACHING EXPERIMENT

Methods and Procedure

Soil columns were set up in the laboratory in cylindrical, transparent plastic tubes, 17 inches high and 1.7 inches in diameter. The bottom of the tubes were plugged by two filter papers enveloped by two sheets of cheese cloth fixed around the outside of the tubes with sticker tape. The tubes, dipped in a funnel, were held vertically with a burette clamp. Fine sand was used to fill the tube up to within two inches from the top. Rock phosphate and treble superphosphate were used as phosphorus sources in this work in amount equivalent to 44 and 88 pounds phosphorus per acre (100 and 200 lbs. P_2O_5/acre) from rock phosphate and 14.5, 29, 44, and 88 pounds phosphorus per acre (33, 66, 100, and 200 lbs. P_2O_5/acre) from treble superphosphate. The fertilizer was broadcast on the surface of the sand except in the case of 14.5 and 29 pounds phosphorus where definite amount of the fertilizer was suspended in a volumetric flask and shaken for one hour and an aliquot containing the desired amount of phosphorus was sprayed on the surface. The leachate was caught in 250 ml Erlenmeyer flask. The leaching solutions were distilled water, iron chelate, or hydrogen chelate. The chelate concentration was 2 ppm for all the phosphorus levels with an additional concentration of 6 ppm for the 44 and 88 pounds phosphorus levels.

To minimize evaporation, parafilm was used to cover the top of the flask with a hole in the center for the funnel stem; at the same time 250 ml beakers were used as lids for the top of the tubes. The leaching solutions were added slowly to the top of the sand in amounts equal to 200 ml, given as two equal portions of 100 ml each, in the morning and the evening of the same day. From some preliminary work it was found that leaching for four days with the proposed volumes would leach out most of the soluble salts in the columns as indicated by the very low conductivity of leachate on the fourth day. On this basis the columns were leached for four days, and the leachate obtained daily was filtered to eliminate any traces of very fine particles and then weighed.

After weighing the amount of leachate obtained in one day, the conductivity, calcium, pH, and phosphorus were determined. The conductivity was measured on a salt bridge, calcium was determined by the versenate method, and phosphorus was determined by the stannous chloride-molybdate method. For those samples in which chelate had been applied, the iron chelate concentration was determined by the method described by Tiffin et al. (1960).

Due to the fact that the used sand contained some impurities, check blanks were designed where the leaching solutions were used in the same manner on the sand, and the same determinations described above were made.

In all the work reported, each treatment consisted of two replications.

To cover the question of phosphorus mobility in the soil profile as affected by the different leaching solutions, a suitable soil sampler was devised. The sampler was initially a table spoon in which the broad end was first bent forward perpendicular to the stem and then the further half of the spoon cut off. After four days of leaching samples were taken from the surface and depths of 2, 4, 8, and 12 inches. The sand was first dried and then digested in a mixture of HNO_3 and $HClO_4$ and phosphorus was determined by the molybdate-metavanadate method of Jackson (1958). Phosphorus mobility was studied only in the two higher levels of superphosphate treatment (44 and 88 pounds of phosphorus per acre) leached with water or chelates. Both the 2 and 6 ppm level of the chelates were used. Soil cores were taken from the blanks leached with the same leaching solution.

Results

Superphosphate as a phosphorus source

Effect of phosphorus level

Superphosphate was added at rates of 0.0, 14.5, 29.0, 44.0, and 88.0 pounds phosphorus per acre (0, 33, 66, 100, and 200 pounds P_2O_5 per acre) and was leached in the columns by either water or 2 ppm iron chelate or hydrogen chelate for four days. The leachate was analyzed for the conductivity, calcium content, and phosphorus content and the results will be discussed under those headings.

Conductivity.--The data on the conductivity measurements for the different treatments for the four days are given in table 8; the data represented by figure 1 are for the sums of the four days. Since the weights of the leachates were not the same, the actual measurements were converted to a percentage basis by multiplying the measurement obtained by the weight of the leachate, then dividing by one hundred. It is thought that such standardization would provide a more logical basis for comparison.

The data do not point out any difference between the leaching solutions in maintaining different conductivity. It could be due to

Table 8. The adjusted conductivity values[a] of the leachate extracted in four days by water and 2 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[b]	Leaching solution[c]	Leaching period, days				
		1	2	3	4	Sum
				micromhos		
0	1	30	5	0	0	35
	2	30	6	0	0	36
	3	32	10	0	0	42
14.5	1	44	12	1	0	57
	2	44	9	0	0	53
	3	42	11	3	1	57
29	1	55	13	2	3	73
	2	52	11	0	0	63
	3	53	12	1	1	67
44	1	73	10	0	0	83
	2	72	8	0	0	80
	3	76	15	0	1	92
88	1	110	17	1	0	118
	2	111	16	0	0	117
	3	111	18	5	0	124

[a] Actual conductivity times the weight of leachate divided by 100.
[b] Pounds per acre.
[c] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA.

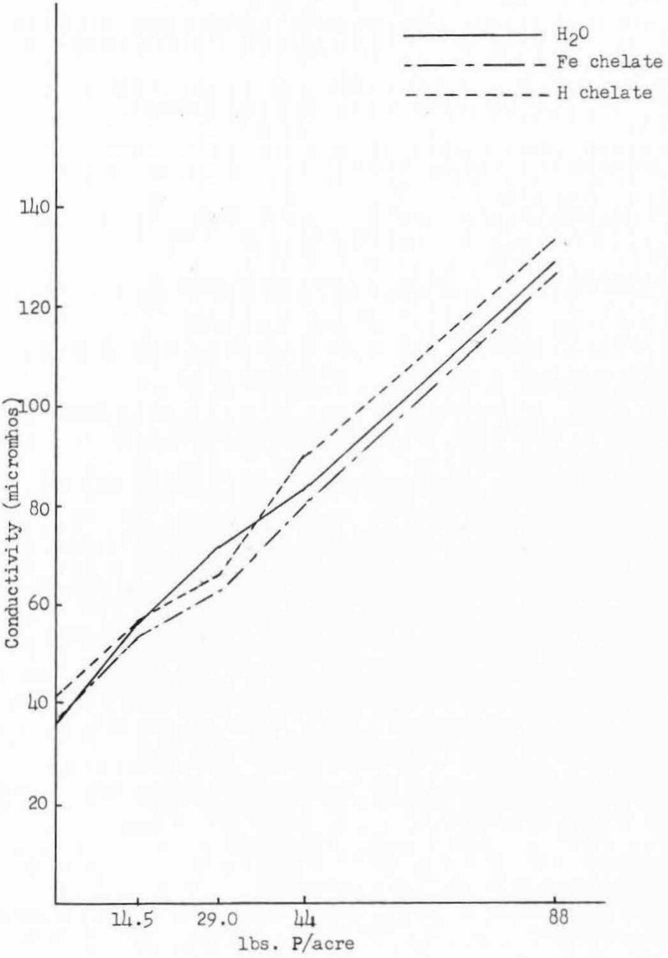

Figure 1. The adjusted conductivity of the total leachate collected in four days as affected by superphosphate level and leaching solution (water and 2 ppm chelate).

the concentrations used being dilute enough to develop any difference, or it might be the measurement itself, too rough to be affected by the used concentrations.

Calcium.--Table 9 reports the data for the calcium content obtained in the leachate for the different treatments in the four periods. The calcium data for the sum of all four days leachings are given in figure 2. From the table it is easily seen that calcium content was not affected by the different leaching solution in the blank and low fertilizer level treatments. As the level of fertilizer increased there was a clear effect of chelate on the calcium removed in the leachate. Both iron chelate and hydrogen chelate decreased the content compared to the non-chelate treatment. Hydrogen chelate decreased the calcium content more than did iron chelate.

Phosphorus.--Table 10 gives the data for the phosphorus content for the four periods of leaching, and figure 3 shows the phosphorus data for the sum of the four periods. In the experiment as a whole, leaching solutions did not show up with a significant response. However, there has been such response within the levels of superphosphate used. In the case of the blank treatment, the differences were almost negligible. With increasing the fertilizer level the difference began to appear. Leaching with both iron or hydrogen chelate removed more phosphorus than did water.

Effect of chelate concentration

In the first part of this experiment, several levels of phosphorus from superphosphate have been leached with water and chelate at one concentration (2 ppm). The purpose of this phase of the experiment was to investigate the effect of chelate averaged over the different levels

Table 9. Calcium content of the leachate extracted in four days by water and 2 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[a]	Leaching solution[b]	Leaching period, days				Sum
		1	2	3	4	
				milligrams		
0	1	2.7	1.7	1.5	1.5	7.4
	2	2.7	1.6	1.6	1.4	7.3
	3	2.1	1.1	0.9	1.1	5.2
14.5	1	10.8	2.4	1.8	1.8	16.9
	2	8.1	2.5	1.8	1.7	14.2
	3	9.5	1.9	1.3	1.2	13.9
29	1	14.2	2.6	1.9	1.6	20.3
	2	9.0	2.3	1.9	1.2	14.4
	3	9.1	2.1	1.7	1.6	14.5
44	1	23.9	2.2	1.9	1.5	29.5
	2	20.7	2.0	1.6	1.6	25.4
	3	12.4	1.7	1.3	1.1	21.9
88	1	27.8	2.5	2.1	1.8	34.1
	2	23.1	2.5	1.8	1.6	30.5
	3	18.2	2.0	1.5	1.3	26.5

[a] Pounds per acre.
[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA.

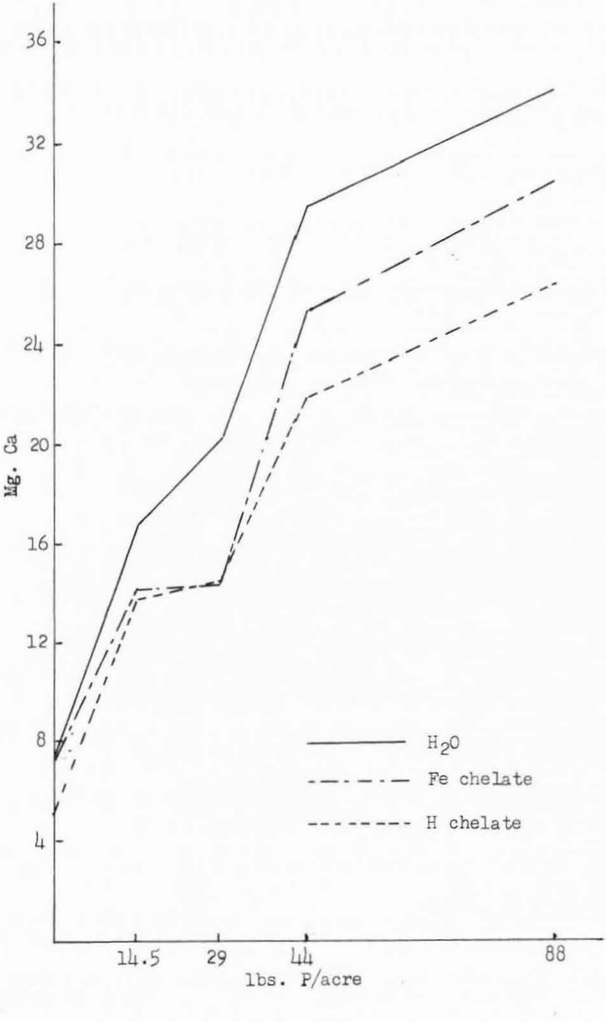

Figure 2. Calcium content of the total leachate collected in four days as affected by superphosphate level and leaching solution (water and 4 ppm chelate).

Table 10. Phosphorus content of the leachate extracted in four days by water and 2 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[a]	Leaching solution[b]	Leaching period, days				Sum
		1	2	3	4	
				milligrams		
0	1	0.12	0.16	0.13	0.13	0.54
	2	0.13	0.15	0.14	0.12	0.53
	3	0.14	0.14	0.15	0.12	0.55
14.5	1	2.44	0.33	0.25	0.18	5.19
	2	5.35	0.37	0.27	0.19	5.68
	3	3.78	0.38	0.25	0.19	4.91
29	1	9.77	0.53	0.34	0.22	10.84
	2	9.95	0.50	0.33	0.21	10.96
	3	8.65	0.57	0.30	0.24	9.75
44	1	12.65	0.46	0.32	0.22	13.65
	2	12.60	0.46	0.32	0.22	14.30
	3	14.90	0.45	0.31	0.21	15.80
88	1	25.90	0.78	0.49	0.34	26.85
	2	25.35	0.68	0.45	0.32	26.70
	3	25.56	0.84	0.55	0.28	29.35

[a] Pounds per acre.

[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA.

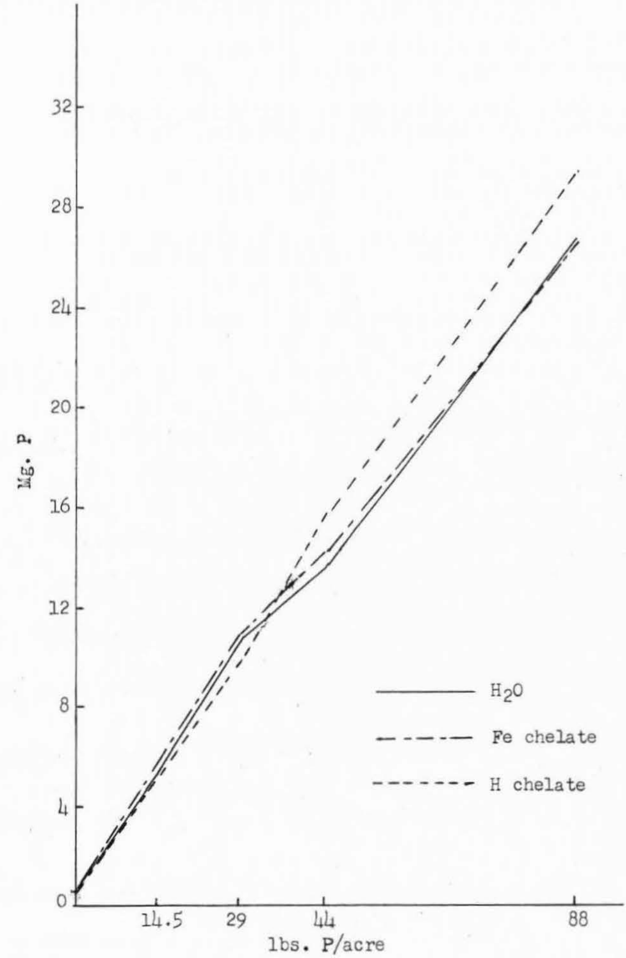

Figure 3. Phosphorus yield of the total leachate collected in four days as affected by superphosphate level and leaching solution (water and 2 ppm chelate).

of the fertilizer. The phosphorus levels were fixed at 0, 44, and 88 pounds of phosphorus from superphosphate while the leaching solution varied through a wide range. The solutions used were water, iron chelate at 2 and 6 ppm, and hydrogen chelate at 2 and 6 ppm.

Conductivity.--Table 11 gives the data for the adjusted conductivity values obtained in the four leaching periods and figure 4 shows the sum of the four periods for the water and chelate at the higher concentration (6 ppm). It is seen that the high chelate concentration of the leaching solutions increased the conductivity. This gives the impression, although probably not justified, that chelate by one or more mechanisms has increased the solubility of some salts which maintained not only a higher conductivity in the first period, but also in the last periods.

Calcium.--Calcium content of four leachates is given in table 12. Figure 5 shows the total calcium obtained with the higher concentrations of chelate and water. The results of this part are similar to those obtained with lower concentration of chelate. Hydrogen chelate yielded less calcium in the leachate than did water or iron chelate treatments. There is just one point to be noticed here concerning the high calcium content of leachate using high chelate concentration. If calcium is retained by the chelate molecule, it would be logical to expect less calcium in the leachate when the more concentrated chelates were used. This is opposite to the results. This discrepancy could be proved to be unreasonable by assuming that the high concentration of chelate has a solubilizing effect on the fertilizer salts which increased the cations in the medium and in the leachate. The solubilizing effect of the high chelate concentration is supported by the fact that these high concentrations yielded higher conductivity.

Table 11. The adjusted conductivity values[a] of the leachate extracted in four days by water and 2 and 6 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[b]	Leaching solution[c]	Leaching period, days 1	2	3	4	Sum
				micromhos		
0	1	30	5	0	0	35
	2	30	6	0	0	36
	3	31	10	0	0	41
	4	29	4	2	0	35
	5	33	5	2	0	40
44	1	73	10	0	0	83
	2	72	8	0	0	80
	3	76	15	0	0	92
	4	74	14	5	0	93
	5	75	19	11	3	107
88	1	110	17	1	0	118
	2	111	16	0	0	117
	3	111	18	5	0	124
	4	113	16	4	1	134
	5	114	18	6	3	141

[a] Actual conductivity times the weight of the leachate divided by 100.

[b] Pounds per acre.

[c] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

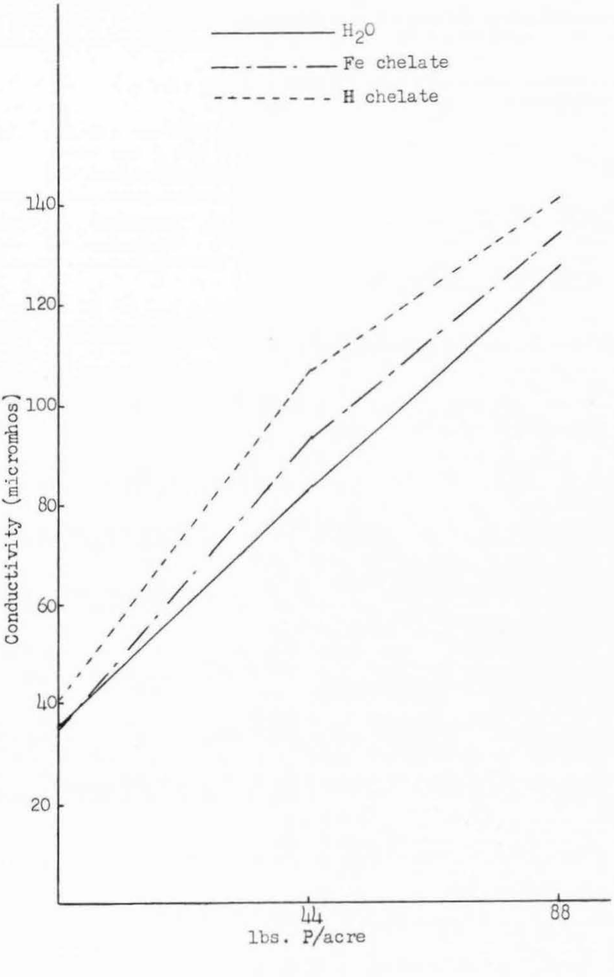

Figure 4. The adjusted conductivity of the total leachate collected in four days as affected by superphosphate level and leaching solution (water and 6 ppm chelate)

Table 12. Calcium content of the leachate extracted in four days by water and 2 and 6 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[a]	Leaching solution[b]	Leaching period, days 1	2	3	4	Sum
				milligrams		
0	1	2.7	1.7	1.5	1.5	7.4
	2	2.7	1.6	1.6	1.4	7.3
	3	2.1	1.1	0.9	1.1	5.2
	4	2.6	1.9	1.5	1.6	7.6
	5	2.1	1.4	1.3	1.3	6.1
44	1	23.9	2.2	1.9	1.5	29.5
	2	20.7	2.0	1.6	1.6	25.4
	3	12.4	1.7	1.3	1.1	21.9
	4	20.6	2.1	1.7	1.6	26.0
	5	15.5	1.8	1.5	1.2	20.7
88	1	27.8	2.5	2.1	1.8	34.1
	2	23.1	2.5	1.8	1.6	30.5
	3	18.2	2.0	1.5	1.3	26.5
	4	27.6	2.8	2.0	1.6	34.0
	5	24.4	2.9	1.8	1.5	28.6

[a] Pounds per acre.

[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

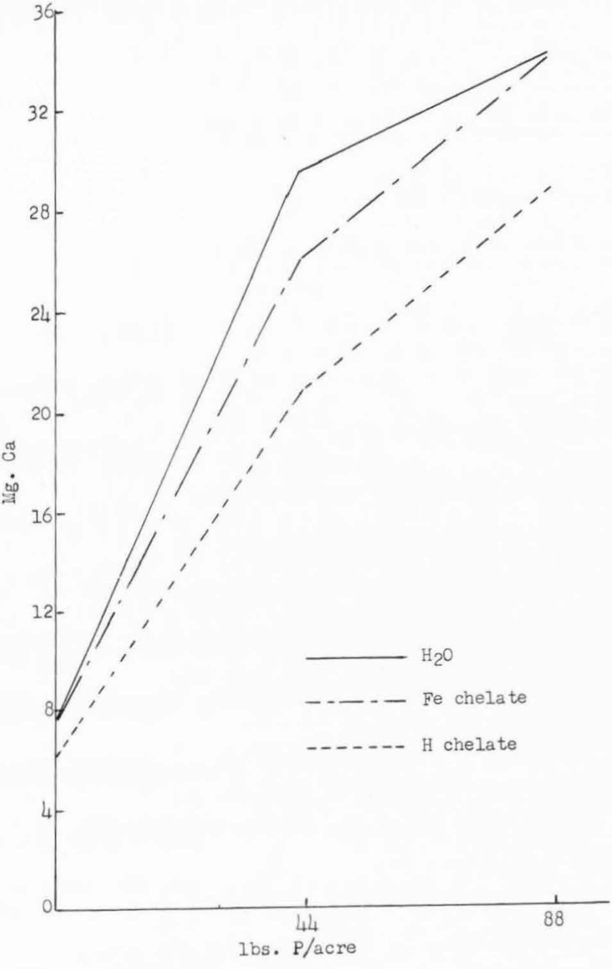

Figure 5. Calcium content of the total leachate collected in four days as affected by superphosphate level and leaching solution (water and [illegible] chelate).

Phosphorus.--Figure 6 shows the total yield of phosphorus (sum of the four leachings) by using higher concentration of chelate and water and table 13 gives the phosphorus content in each period. In the low fertilizer level all chelate treatments yielded more phosphorus than the non-chelate treatment. Iron chelate at the higher concentration yielded more phosphorus than at the lower concentration. That is expected if the assumption of mixed chelate formation is correct. In the case of hydrogen chelate the two chelate concentrations were not significantly different at the 44 pounds of phosphorus level. At the highest phosphorus level the highest chelate concentration gave a significant increase in phosphorus.

Total phosphorus and recovery

Phosphorus content in the leachate of the four periods under the different treatments was summed to give the total phosphorus that was leached out of the columns. The results are shown in the first part of table 14. Phosphorus obtained from the blank treatment was negligibly small and to some extent was not an indicator to the effect of the leaching solution, so it is not reported as a treatment.

From the table we can see that the previous conclusions concerning the phosphorus level and chelate level still hold. Generally the phosphorus yield in chelate treatments was greater than the non-chelate treatment. This effect increases to the maximum under a combination of a certain chelate form and phosphorus level. See also figure 7.

Another basis for comparing the effect of leaching solutions on phosphorus solubility can be obtained by calculating the percentage recovery. This basis would provide the knowledge of how much of the applied phosphorus was obtained out of the columns as affected by

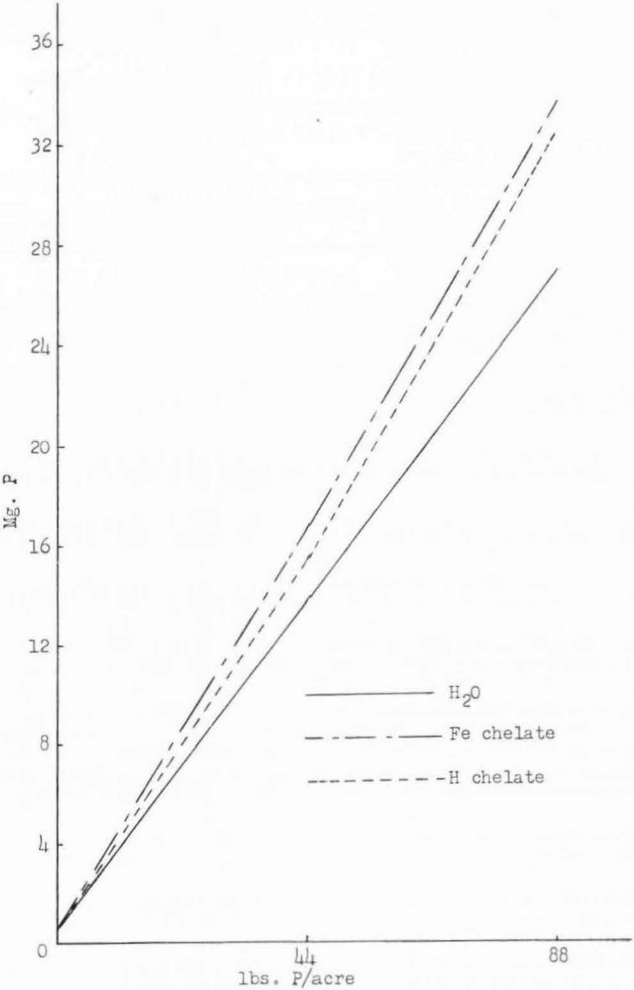

Figure 6. Phosphorus yield of the total leachate collected in four days as affected by superphosphate level and leaching solution (water and 6 ppm chelate).

Table 13. Phosphorus content of the leachate extracted in four days by water and 2 and 6 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[a]	Leaching solution[b]	Leaching period, days				
		1	2	3	4	Sum
				milligrams		
0	1	0.12	0.16	0.13	0.13	0.54
	2	0.13	0.15	0.14	0.12	0.53
	3	0.14	0.14	0.15	0.12	0.55
	4	0.15	0.16	0.15	0.14	0.58
	5	0.15	0.16	0.14	0.12	0.57
44	1	12.65	0.46	0.32	0.22	13.65
	2	13.35	0.46	0.31	0.24	14.30
	3	14.90	0.45	0.31	0.21	15.80
	4	15.60	0.50	0.32	0.22	16.60
	5	14.30	0.56	0.32	0.23	15.50
88	1	25.90	0.78	0.49	0.34	26.85
	2	25.35	0.68	0.45	0.32	26.70
	3	25.55	0.84	0.55	0.28	29.35
	4	31.90	0.81	0.53	0.35	33.55
	5	30.50	0.84	0.54	0.43	32.30

[a] Pounds per acre.

[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

Table 14. Total phosphorus and percentage of phosphorus recovery in the leachate with water and 2 and 6 ppm chelate when the phosphorus source was superphosphate

Phosphorus level[a]	Leaching solution[b]	Total[c]			Recovery[d]		
		Replication			Replication		
		1	2	Mean	1	2	Mean
		milligrams			percent		
14.5	1	4.99	5.39	5.19	73.5	79.4	76.5
	2	5.88	5.47	5.68	86.7	80.6	83.7
	3	5.49	4.32	4.91	80.9	63.7	72.3
29.0	1	10.74	10.93	10.84	71.3	72.8	72.1
	2	10.82	11.09	10.96	79.8	81.7	80.8
	3	9.50	10.00	9.75	70.1	73.7	71.8
44.0	1	13.50	13.80	13.65	66.6	68.1	67.4
	2	14.70	13.90	14.30	72.6	68.6	70.6
	3	15.90	15.70	15.80	78.5	77.5	78.0
	4	15.90	17.30	16.60	78.5	85.4	82.0
	5	15.40	15.50	15.45	76.0	76.5	76.3
88.0	1	26.90	26.80	26.85	66.4	66.2	66.3
	2	27.80	25.60	26.70	68.6	63.2	65.9
	3	26.70	32.00	29.35	65.9	79.0	72.5
	4	36.72	30.40	33.55	90.7	75.0	82.9
	5	32.70	31.90	32.30	79.2	78.8	79.0

[a] Pounds per acre.

[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

[c] Sum of phosphorus content in four leachates.

[d] Sum of phosphorus content in leachate divided by the phosphorus added as fertilizer.

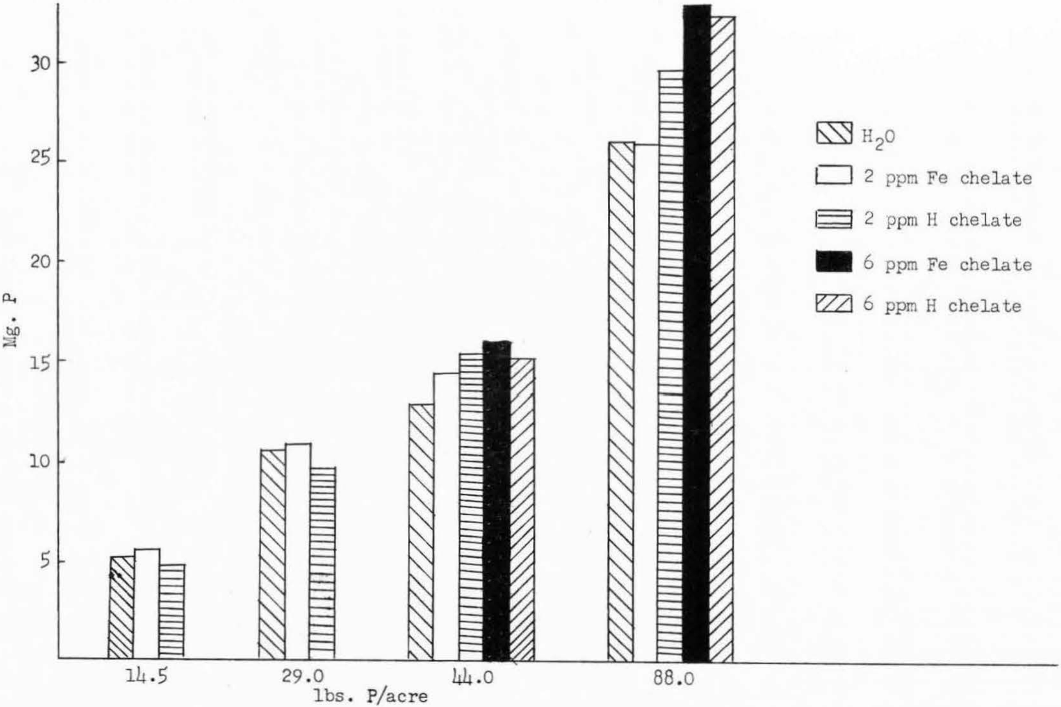

Figure 7. Total phosphorus contained in the leachate collected in four days upon leaching superphosphate with water and the indicated concentration of chelate.

leaching solution as well as fertilizer level. It was calculated by dividing the total phosphorus content in the leachates of the four periods by the amount of the fertilizer applied to the columns. The results are shown in the second part of table 14. It was not possible to analyze statistically the whole data of this part as one unit because the assymmetric nature of the experiment where two out of the four fertilizer levels did not receive the higher concentration of chelates. Although the statistical analysis was done in two steps, the interpretation will be stated in one step depending on the pattern shown in figure 8.

From the graph we see in water treatments, the recovery decreased by increasing phosphorus level. In the low concentration of iron chelate (2 ppm) the efficiency was at a maximum in the lowest fertilizer level and decreased gradually with increasing phosphorus level. The high concentration of iron chelate was the most effective in increasing the recovery. The low concentration of hydrogen chelate (2 ppm) gave a gradual increase in recovery with increasing fertilizer level up to 44 pounds of phosphorus and then it showed a decrease at the 88-pound level. In the high concentration we find that increasing the chelate level did not improve the recovery in the 44-pound level while it did when the fertilizer level had been doubled. It is clear that the recovery obtained by this chelate was controlled by some relation between the chelate level and the cations level which is limited by the fertilizer level.

Phosphorus mobility

The sand samples taken from the columns after leaching for four days were analyzed for total phosphorus. As the purpose of this work

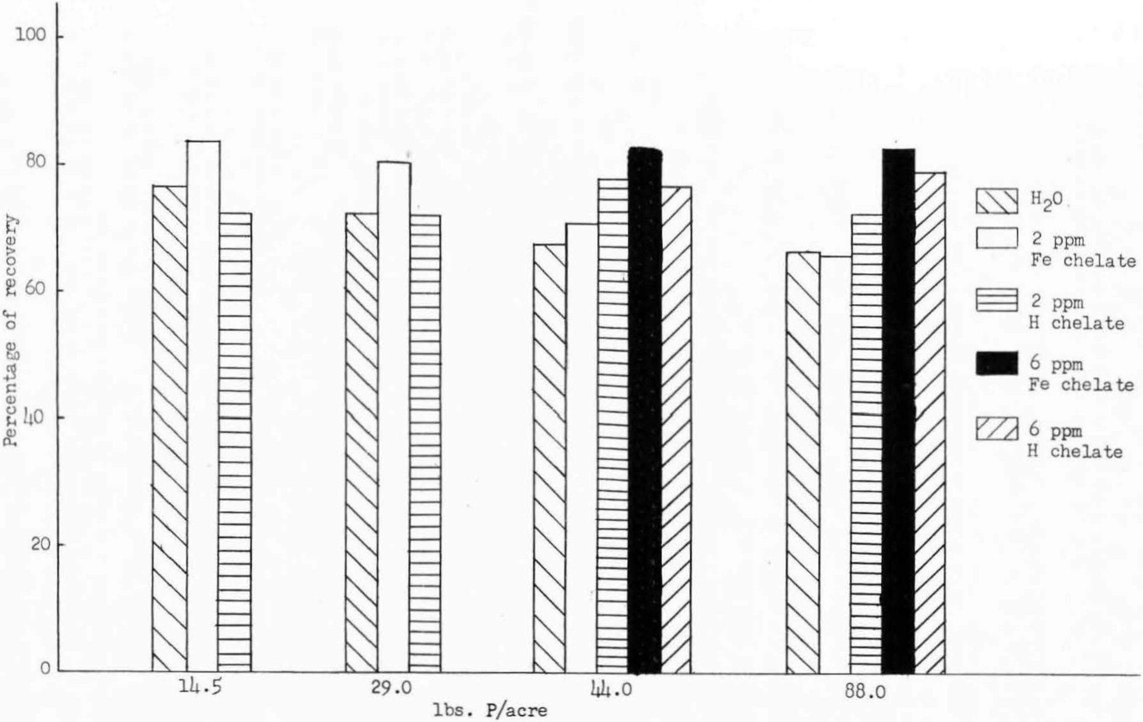

Figure 8. Percentage of the phosphorus recovered in the leachate as affected by superphosphate level when leached with water or two concentrations of chelate.

was to study the effect of the treatments on phosphorus mobility in the profile rather than their effect on phosphorus availability, it was thought that total phosphorus rather than available phosphorus might give a more comprehensive idea. The results of two replications are averaged in table 15. From the table it is clear that the different leaching treatments had little effect on the mobility of the initial phosphorus of the sand as indicated by small differences among different depths for the blank phosphorus level.

The most effective leaching solution for the intermediate phosphorus level (44 pounds per acre) on the surface was water, which differs significantly from any other leaching solution. If the 2-inch depth rather than the surface is regarded, it can be seen that the phosphorus content at this depth was lower in the water treatment than in any other treatment. The interesting point, however, is the phosphorus gradient from the surface to this depth. In water treatment the gradient was much sharper than for any other treatment. In the lower depth of the profile the differences between the treatments reached the minimum.

The experiment was conducted on sand that contained some initial phosphorus. The solutions did not affect significantly the movement and redistribution of this initial phosphorus through the columns, but in the overall, the solution effects were not identical. So the discussion covering this movement of phosphorus from the fertilizer as affected by the different leaching solutions on the basis of the adjusted means seems to be logical. The adjusted means were obtained by subtracting the amount of phosphorus in the blank at each given depth from the corresponding amount of phosphorus at that depth under

Table 15. Phosphorus recovered[a] at the various depths in sand column when the phosphorus source was superphosphate

Depth	Phosphorus level[b]														
	0					44					88				
	Leaching solutions[c]					Leaching solutions					Leaching solutions				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
inches	milligrams per gram sand														
0	0.03	0.05	0.07	0.06	0.06	0.04	0.07	0.08	0.08	0.07	0.09	0.08	0.09	0.10	0.13
2	0.06	0.06	0.07	0.06	0.06	0.10	0.09	0.12	0.12	0.13	0.10	0.13	0.13	0.14	0.13
4	0.05	0.07	0.07	0.06	0.07	0.09	0.11	0.11	0.09	0.11	0.12	0.12	0.12	0.10	0.11
8	0.07	0.07	0.07	0.06	0.06	0.09	0.10	0.10	0.08	0.10	0.10	0.12	0.12	0.11	0.10
12	0.05	0.06	0.06	0.06	0.07	0.08	0.09	0.08	0.07	0.07	0.09	0.10	0.10	0.09	0.08
	Adjusted means[d]														
0	--	--	--	--	--	0.010	0.020	0.010	0.020	0.005	0.060	0.030	0.020	0.035	0.020
2	--	--	--	--	--	0.045	0.030	0.050	0.060	0.045	0.045	0.070	0.055	0.075	0.070
4	--	--	--	--	--	0.040	0.035	0.040	0.025	0.035	0.070	0.055	0.050	0.045	0.040
8	--	--	--	--	--	0.020	0.040	0.030	0.025	0.035	0.035	0.045	0.045	0.040	0.035
12	--	--	--	--	--	0.030	0.030	0.025	0.015	0.000	0.035	0.040	0.040	0.030	0.010

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[a] Average of two replications.

[b] Pounds per acre.

[c] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

[d] Obtained by subtracting the phosphorus found in the blank from that found in the treated sand.

the fertilized treatment. The results are presented in the lower part of table 15 and figures 9 through 12. Under this condition it seems that a line could be drawn to separate the interaction of leaching solutions with each phosphorus level that has been used. For the surface of the intermediate level (44 pounds phosphorus per acre), hydrogen chelate at the low concentration was as effective as water and more efficient at the higher concentration. The amount retained in iron chelate treatment did not increase when the chelate concentration was increased.

In the 2-inch depth it could be concluded that solutions that were most effective in leaching surface gave the highest phosphorus analyses. The explanations of this phenomenon could stand on two bases. First, increasing the leachate saturation with phosphorus makes the amount of phosphorus in contact with sand higher, consequently a higher fixation. The relatively high analysis in the higher concentration of hydrogen is due to that saturation plus a possibility of fixing the mixed chelate molecule, including phosphorus molecule on the sand surface. In the case of the higher concentration of iron chelate, it could be due to either chelate fixation or precipitation of formerly formed iron phosphate or both. The difference among solutions decreases with increasing depth except in the case of the last depth of the high hydrogen chelate concentration which did not show any phosphorus. It is believed that this is no more than either an experimental error or a complication in calculating the adjusted means.

The analysis of the surface for the water leached treatment for the higher phosphorus level (88 pounds per acre) seems to be odd but it could be said that the high level of P inaugurated the efficiency of chelate in leaching through the formation of mixed chelate. There was

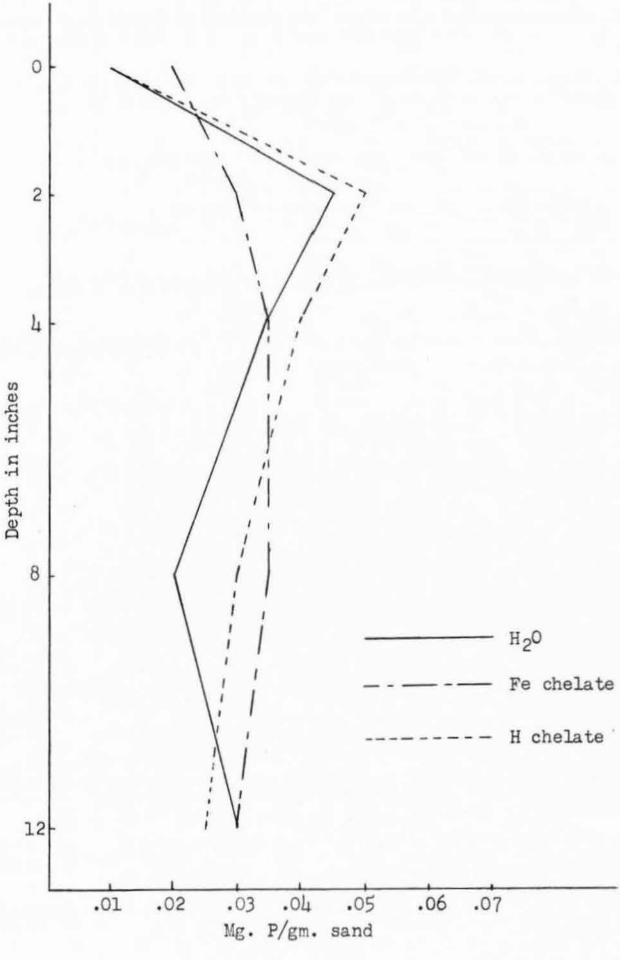

Figure 9. Depth distribution of phosphorus from that applied as superphosphate at a rate of 44 pounds phosphorus per acre as affected by leaching with water and 2 ppm chelate.

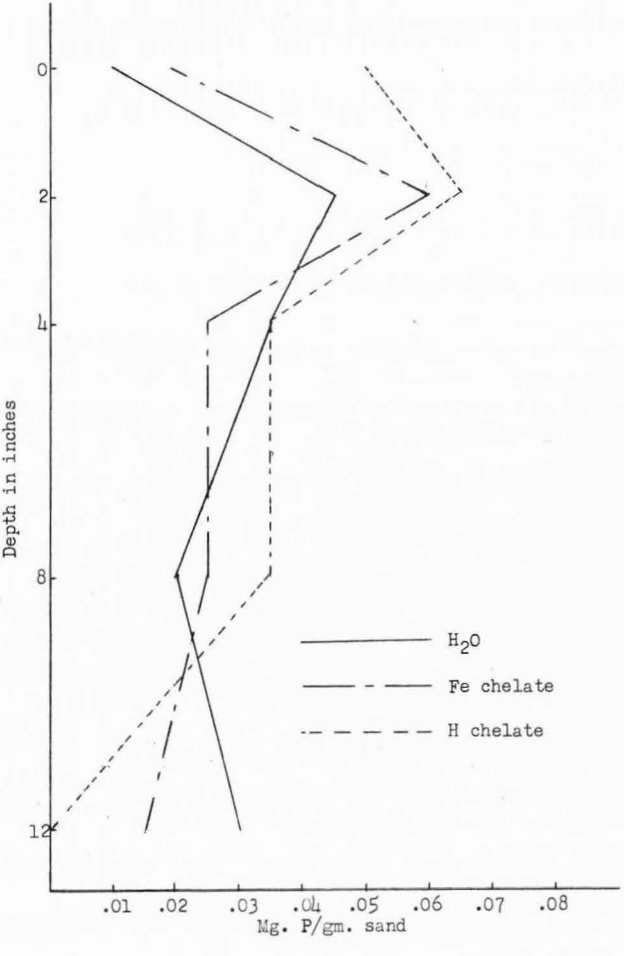

Figure 10. Depth distribution of phosphorus from that applied as superphosphate at a rate of 44 pounds phosphorus per acre as affected by leaching with water and 6 ppm chelate.

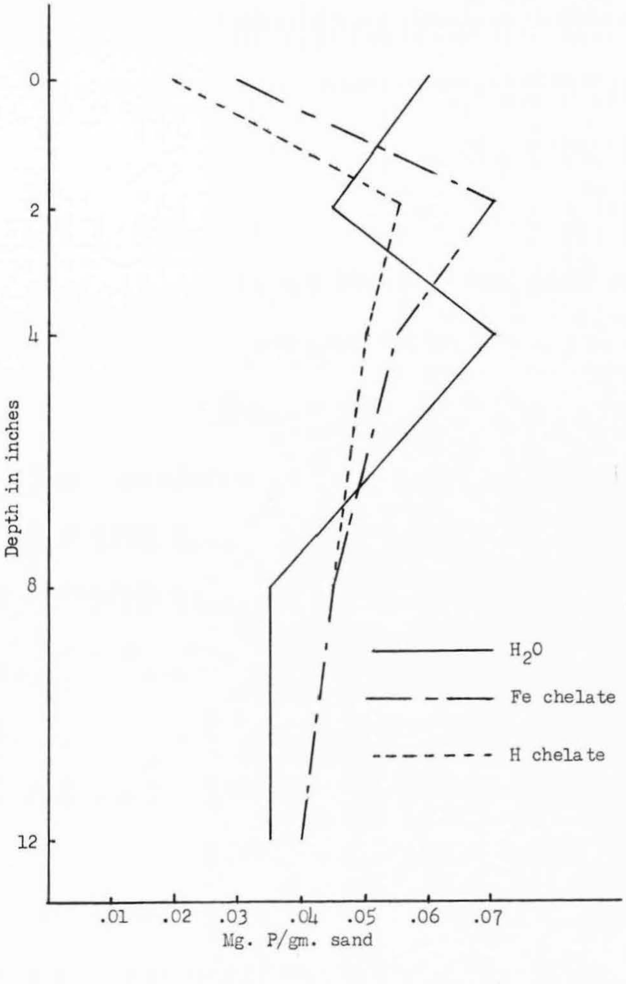

Figure 11. Depth distribution of phosphorus from that applied as superphosphate at a rate of 88 pounds phosphorus per acre as affected by leaching with water and 2 ppm chelate.

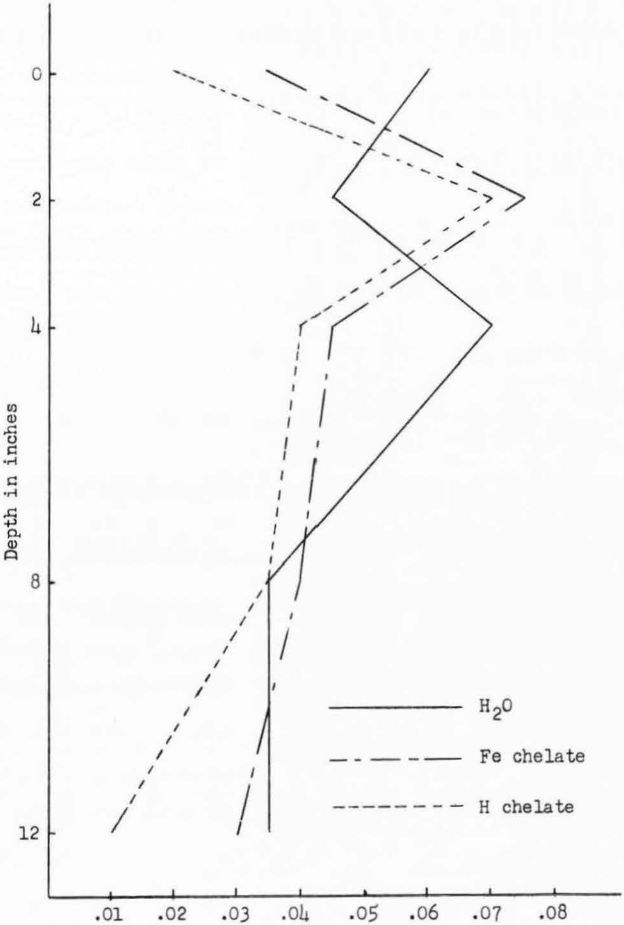

Figure 12. Depth distribution of phosphorus from that applied as superphosphate at a rate of 88 pounds phosphorus per acre as affected by leaching with water and 6 ppm chelates.

a somewhat greater amount of phosphorus in the surface associated with the higher concentration of iron chelate when compared with the lower iron chelate concentration which could be due to the formation of iron phosphate.

In the 2-inch depth, water treatment contains the least analysis due to the inadequate leaching of the surface while the higher concentration of iron chelate treatment contains the highest analysis. This may be due to the precipitation of iron phosphate. In the 8-inch depth the difference among the treatment reaches a minimum. This could be attributed to the action of sand in sieving the chelate molecules out of the solution, leaving just water as a leaching solution.

Rock phosphate as a phosphorus source

To investigate the effect of chelates on increasing the availability of phosphorus from an insoluble source, rock phosphate has been used as a phosphorus source.

This work was originally designed to use the same phosphorus levels that were used in the superphosphate experiment. The results obtained for the low levels were not distinguishable from the blank treatment. So the work continued using the two highest phosphorus levels, 44 and 88 pounds phosphorus per acre. Since the treatments were symmetrical where all leaching solutions were applied to all phosphorus levels, the effect of chelate concentration and phosphorus level will be presented together.

Effect of chelate and phosphorus level

Conductivity.--Table 16 shows the effect of the different leaching solutions on the conductivity pattern in the four periods. From the table it can be seen that rock phosphate did not affect the conductivity

Table 16. The adjusted conductivity values[a] of the leachate extracted in four days by water and 2 and 6 ppm chelate when the phosphorus source was rock phosphate

Phosphorus level[b]	Leaching solution[c]	Leaching period, days				Sum
		1	2	3	4	
				micromhos		
0	1	30	5	0	0	35
	2	30	6	0	0	36
	3	31	10	0	0	41
	4	29	4	2	0	35
	5	33	5	2	0	40
44	1	33	8	0	0	41
	2	31	3	0	0	34
	3	33	11	1	1	46
	4	32	9	4	1	46
	5	31	7	5	0	42
88	1	35	6	2	0	42
	2	31	4	0	0	34
	3	34	11	3	0	48
	4	31	6	3	3	42
	5	37	6	3	1	46

[a] Actual conductivity times the weight of leachate divided by 100.

[b] Pounds per acre.

[c] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

to the same extent that superphosphate did. This was expected because of the difference in solubility of both materials. If there is any effect of chelate on solubilizing rock phosphate, it should be observed in the latter periods where most of the soluble salt has been flushed out of the columns in the first period. In the latter periods, iron chelate in the lower concentration has decreased the conductivity while it increased it at the higher concentration. In the case of hydrogen chelate, the situation was reversed, where the lower concentration gave higher conductivity.

Calcium.--Table 17 presents the calcium content of the leachates in the four periods. If the calcium content as affected by the leaching solutions, as averaged over all the fertilizer level was inspected, it could be seen that both chelate forms yielded less calcium than the water treatment. The higher concentration of iron chelate yielded the same amount of calcium as did that of the lower concentration. The case is exaggerated upon using hydrogen chelate where the higher concentration of the chelate gave more calcium than the lower concentration. These results have some support in the data reported for superphosphate.

The relative effects of the leaching solutions can be examined also by the increase in the fertilizer level. Increasing the phosphorus level from 44 to 88 pounds has increased the amount of calcium removal by the magnitude that resulted increasing the level from 0 to 44 pounds in water as a leaching solution. When iron chelate was used in the lower concentration, there was no increase in calcium upon increasing the phosphorus level from 44 to 88 pounds.

By using iron chelate in the high concentration, there has been an increase, though far less than the corresponding increase in water

Table 17. Calcium content of the leachate extracted in four days by water and 2 and 6 ppm chelate when the phosphorus source was rock phosphate

Phosphorus level[a]	Leaching solution[b]	Leaching period, days				Sum
		1	2	3	4	
		milligrams				
0	1	2.7	1.7	1.5	1.5	7.4
	2	2.7	1.6	1.6	1.4	7.3
	3	2.1	1.1	0.9	1.1	5.2
	4	2.6	1.9	1.5	1.6	7.6
	5	2.1	1.4	1.3	1.3	6.1
44	1	3.4	2.0	1.8	1.8	8.7
	2	2.0	1.4	1.6	1.6	7.3
	3	2.8	1.2	1.0	1.2	6.1
	4	2.8	2.0	1.7	1.3	7.8
	5	2.2	1.4	1.7	1.4	6.7
88	1	3.7	1.7	1.9	1.7	9.0
	2	2.8	1.6	1.5	1.5	7.4
	3	3.0	1.2	1.2	1.2	6.6
	4	3.1	1.9	1.7	1.8	8.4
	5	2.4	1.4	1.4	1.3	6.5

[a] Pounds per acre.

[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

treatment. In the case of hydrogen chelate, the lower chelate concentration gave about half of the increase in going from 44 to 88 pounds of phosphorus as was obtained from 0 to 44. The higher concentration reduced the calcium content when the 88 pounds were compared with the 44-pound level.

Phosphorus.--Table 18 reports phosphorus content of the four periods. As can be seen from the table, there has been a small difference among the treatments, both the leaching solutions and phosphorus level. The statistical analysis reveals that there is a difference due to the leaching solutions and phosphorus level.

With respect to phosphorus level, the increase from 44 to 88 pounds yielded about one-half of the increase resulted from an increase from 0 to 44 pounds. This is expected in salts with limited solubility such as rock phosphate according to the solubility product principle, where a certain amount of the salt dissolves in a certain volume of a solution. With respect to the different leaching solutions, the lower concentration of iron chelate has decreased significantly the phosphorus yield, while the high concentration of hydrogen chelate increased it.

Discussion

Phosphorus solubility

One reason in explaining the effect of chelate in increasing phosphorus solubility is its effect on decreasing calcium content of the solution. The decreased content of calcium in the leachate when iron chelate was used can be explained by two possible mechanisms. The first includes an exchange between the iron molecule in the chelate structure for the calcium ion in the solution. Measurement of iron chelate concentration in the leachate (Tiffin et al., 1960) was inconclusive as the

Table 18. Phosphorus content of the leachate extracted in four days by water and 2 and 6 ppm chelate when the phosphorus source was rock phosphate

Phosphorus level[a]	Leaching solution[b]	Leaching period, days				Sum
		1	2	3	4	
				milligrams		
0	1	0.12	0.16	0.13	0.13	0.54
	2	0.13	0.15	0.14	0.12	0.53
	3	0.13	0.14	0.15	0.12	0.55
	4	0.14	0.16	0.15	0.14	0.58
	5	0.15	0.16	0.14	0.12	0.57
44	1	0.13	0.15	0.16	0.14	0.58
	2	0.13	0.14	0.14	0.12	0.54
	3	0.15	0.15	0.17	0.11	0.59
	4	0.14	0.16	0.14	0.13	0.56
	5	0.16	0.18	0.15	0.12	0.61
88	1	0.13	0.16	0.17	0.13	0.59
	2	0.13	0.13	0.13	0.13	0.52
	3	0.14	0.15	0.16	0.13	0.58
	4	0.16	0.16	0.15	0.13	0.59
	5	0.17	0.17	0.15	0.14	0.63

[a] Pounds per acre.

[b] 1 = water; 2 = 2 ppm Fe-EDDHA; 3 = 2 ppm H-EDDHA; 4 = 6 ppm Fe-EDDHA; 5 = 6 ppm H-EDDHA.

leachate of all treatments gave about the same response to the measurement. However, it is felt that the exchange of iron from the iron chelate for calcium cannot count for the significant decrease in calcium content in this case because of the well known fact that iron forms a more stable chelate than calcium. The second point that might explain the low calcium content in the leachate from the iron chelate treatment is the formation of mixed chelate (Thompson and Lorass, 1962; Bongucki, 1962; and Martell and Calvin, 1952). A mixed chelate is where the metal in the chelate structure is surrounded by two or more different active groups. As will be seen later, iron chelate has reacted with phosphate to form the mixed chelate. On such basis, it is reasonable to conclude that the more mixed chelate formation, the more charge inequilibrium on the molecule in such direction that would be able to retain more positive ions as calcium, which is available in the medium. The calcium content in the hydrogen chelate treatment has decreased relatively to iron chelate, by increasing the fertilizer level.

Hydrogen chelate was inferior to iron chelate in the phosphorus yield in the lower levels of fertilizers because hydrogen chelate is no more than a structureless chelating agent, which assumes the chelate structure only by introducing cations such as iron or calcium. Adding more fertilizer adds more cations to the solution and the chelating agent might be changed to a chelate capable of forming the mixed chelate, consequently increasing the phosphorus yield. At the higher phosphorus levels hydrogen chelate solubilized more phosphorus than did iron chelate, both at the lower concentration. This could be due to the decreased efficiency of iron chelate as a phosphorus carrier beyond a certain point of phosphorus saturation, after which any excess phosphorus in the chelate structure might react with the iron cation to form the insoluble iron phosphate. It could be due to the increased efficiency of the hydrogen chelate which has been converted to the chelate structure thereby capable of forming the mixed chelate. Another possibility is the decreased cation (calcium) concentration of the medium more than in the iron chelate treatment. This decrease is brought about by the H chelate becoming Ca chelate and thereby removing the calcium from the solution. By decreasing the calcium the solubility of phosphorus is increased, not only because of eliminating a precipitating factor but also by causing a drop in the pH of the solution. The process of converting a chelating agent in the chelate structure might have a direct effect on the pH. As mentioned by Martell and Calvin (1952), "All metal chelate may be considered as formed by the displacement of one or more usually weak acidic protons of the chelating agent by a metal ion. Thus, the addition of glycine to a solution of cupric salt causes a drop in the pH. The greater the tendency for metals to combine with a given chelating agent, the greater the drop in pH." However, pH measurements in the leachate did not reveal any difference, since all the values ranged very closely about 7, which was the original pH of the leaching solution. Calcium retained by the mixed chelate molecule is expected to be acting here too.

In the high concentration of the hydrogen chelate used with the lower fertilizer level, we have increased the amount of the chelating agent by three times. Meanwhile the cations concentrations did not have an equal increase. In other words, just a part of the chelating agent was converted to the chelate structure, while the rest remained in the form of the chelating agent. On such basis, the amount of phosphorus in the high concentration did not differe from that of the lower concentration. The discussion about cation concentrations just mentioned does not agree with what was revealed by conductivity and calcium measurements, where high concentrations of chelate were associated with higher values. But this does not represent a discrepancy, because it is known that the reaction between chelating agents and metals is molecular rather than ionic reaction. It is known that the molecular reactions require a longer time for reaction to be complete. In the high level of fertilizer, water and the low concentration of chelates gave almost the same values. The relatively low yield for iron chelate in this case could be due to a possible reaction between iron and phosphate, after a saturation point, to form the insoluble iron phosphate. The relatively low yield in the case of hydrogen chelate in this case is not quite understood, although it gave quite high yield in the second and third periods. With respect to the high concentration iron chelate gave a high yield. In this case the chance of forming iron phosphate is not quite possible due to the decrease in phosphorus:chelate ratio. Hydrogen chelate, with enough calcium to form the chelate structure, was more effective than it was in the low fertilizer level.

If phosphorus recovery was regarded, it is seen that with water as leaching solution the recovery percentage decreased by increasing the phosphorus level. The opposite was expected, but this could be correct only on soils with a fairly high phosphorus fixing capacity where the successive phosphorus increments would suffer less fixation due to approaching the point of saturation. But in the case of this work, conducted on sand, it appears that its capacity to fix phosphorus was not great enough to be seriously regarded. Also, its initial phosphorus content might have been enough to satisfy the fixing capacity. So the decrease in recovery could be explained on the basis that the amount of phosphorus leached out by water was limited by the degree of contact between water and the fertilizer in the columns. Jordan et al. (1952) found, using a constant amount of superphosphate, that the downward movement of phosphorus in the soil profile was directly proportional to the amount of irrigation water. It is logical to extract another conclusion from Jordan's work; that is the relative movement of different amounts of phosphorus, using constant amounts of irrigation water, would be greatest at the lower fertilizer level. This was the case in the data on recovery calculation (figure 8) where it is shown that the greater the fertilizer level the less the relative contact between phosphorus and water and therefore, the lower the recovery percentage.

In the low concentration of iron chelate (2 ppm) the efficiency was at a maximum in the lowest fertilizer level and decreased gradually with increasing phosphorus level. Such a pattern can be explained in two ways. The first one is the relation between the solution and the fertilizer which limits the recovery by the degree of the contact, as explained for water treatment. The second reason is the possible reaction of the phosphate group and the iron molecule within the chelate structure to form an insoluble iron phosphate. At the highest level of fertilizer recovery of iron chelate was not significantly different from the water treatment, but the criterion to be considered is the degree of decrease in both cases.

The low concentration of hydrogen chelate (2 ppm) gave a gradual increase in recovery with increasing fertilizer level up to 44 pounds of phosphorus and then it showed a decrease at the 88-pound level. This drop could be thought about through several possibilities. The first one is that there is a maximum efficiency of the chelate in increasing the recovery due to the limited amount of chelate supplied by the low concentration used. A second possibility is the contact effect explained previously for the water treatment. A third possibility is that the recovery might be higher than it is indicated by the average because the range of the two replications was relatively wide.

The high concentration of iron chelate (6 ppm) was very effective in increasing the recovery. The chance of iron phosphate formation in this case is smaller than when the low concentration of the chelate was used. This could be confirmed by checking phosphorus-chelate-recovery relations from the standpoint of phosphorus level and chelate concentration. In the 14.5 and 44-pound treatments, the 2 ppm iron chelate gave 84 and 71 percent recovery. In other words, increasing phosphorus level by three times decreased the recovery by 13 percent. Using 6 ppm and 2 ppm iron chelate in the 44-pound phosphorus level gave phosphorus recovery of 82 and 71 percent, respectively, or increasing the chelate concentration increased the recovery by 11 percent. If the decrease in recovery due to the contact effect is set to be about 2 or 3 percent, we could see that the increase in efficiency of iron chelate, by increasing its concentration by a factor, is equal to the decrease in its efficiency upon raising the amount of fertilizer by the same factor.

In hydrogen chelate treatment we find that increasing the chelate level did not improve the recovery in the 44-pound level while it did when the fertilizer level had been doubled. This supports the assumption that the chelating agent has to be converted first to the chelate structure if any effect of chelate on phosphorus is to be expected. Even if the cations, which are necessary to convert the chelating agent to the chelate structure, are available, the nature of the chelation reaction which is a molecular one, might be a factor in the effect of hydrogen chelate on recovery. This is indicated in the 88-pound level of phosphorus, where iron chelate was superior to the hydrogen chelate, both at the high concentration. That is the result of the slow reaction between cations, which have been available in the medium, and the chelating agent to form the chelate structure.

Phosphorus mobility

The different leaching treatments had little effect on the mobility of the initial phosphorus of the sand. There are two possible reasons why this might be, because the texture of the sand permitted the water to infiltrate fast enough to miss any significant contact with the initial phosphorus. Such contact is necessary to solubilize and leach the phosphorus down. The second factor might be the way the leaching solutions were added to the columns. Although these solutions were added slowly, still they were added from a graduate cylinder. Even if we assume that the solution had a chance to solubilize sand phosphorus, it is easily seen that the flow is sufficiently fast to lead to two consequences. The first is that the degree of contact between the solution and the sand would be equal at any given depth in the column which equalizes the solubilization at these different depths. The second consequence is that due to fast flow any solubilized phosphorus would be leached completely out of the column with no chance to be fixed at deeper levels of the column.

The explanation just given provides a reasonable expectation to leaching with water but chelate treatments require more clarification. The mechanism by which chelate increases phosphorus availability mentioned earlier provides such clarification. To form mixed chelate, in the case of the iron chelate, would require only a soluble phosphorus source that would be already available to share in the groupings that surround the iron atom. As such source is not, at least adequately, available, iron chelate in the two concentrations that were used did not differ from water. In the case of hydrogen chelate, it should be remembered that this material is not more than a closed bag with a lost key. The key here is the cation which unfolds the material to form the chelate structure which is capable of retaining the phosphorus ion among the rings. As such a cation was not available in an amount enough to open the closed bag, the results of hydrogen chelate in both concentrations did not differ significantly from water treatment.

The relatively higher amount of phosphorus in the surface of the chelate treated columns can possibly be explained through two mechanisms. The first, which is common in both Fe chelate and H chelate treatments, is the possible fixation of the mixed chelate which contains phosphorus molecule, on the surface of the sand particle. This phenomenon, though not clear in the case of the low level of chelate, was almost significantly clear in the higher level. This means that by increasing the chelate concentration, and consequently increasing phosphorus mixed inside the chelate structure, the amount of phosphorus retained by the sand surface increased. The second mechanism is special for iron chelate treatment where it could be defended that under conditions where phosphorus and iron get together there is a chance of forming the insoluble iron phosphate. The statistical proof for this mechanism is lacking, but the consistency of results gives the idea.

The adjusted means showed that the amount retained by the sand in the upper depths was greater in the case of iron chelate than for any other treatment. Thus we have support for the assumption of iron phosphate formation. The equal amount of phosphorus under the two concentrations of iron chelate might represent a contradiction to this assumption, but it could be argued that the flow was fast enough so that a complete contact between the chelate and the phosphorus was insufficient to form adequate amount of the insoluble iron phosphate. Gravitational movement of the precipitated iron phosphate to the subsurface is another possibility.

In conclusion to this experiment, it should be mentioned that the depths tested probably were too far apart. Also, with just two replications, one can get only a rough idea of what might be going on concerning the gradient of phosphorus content as related to the soil profile. The results reported here agree, in principle, with that reported by Jordan et al. (1952) where they found that the extent to which phosphorus moved from the surface to the subsurface was directly proportional to the amount of leaching water. In the work reported here, although constant volumes of leaching solution were used, the different levels of phosphorus would change the relation between the volumes and the phosphorus in a way to alter the efficiency of the solutions to mobilize phosphorus. The irregularities of phosphorus gradient from the surface to the bottom of the column has been observed in earlier work by Bouldin and Black (1959). They explain such irregularity as a result of periodic precipitation of phosphorus based on the fact that calcium monohydrogen phosphate ($CaHPO_4$) has a strong tendency to supersaturate; consequently there is a periodic precipitation of phosphorus.

Fixation of chelate in soil has been mentioned by some workers

(Wallace et al., 1955, and Hill-Cottingham, 1957). A straightforward application to this conclusion in the work reported here is not absolutely valid, because it was assumed that the fixation reaction is with the clay fraction of the soil. The soil material used here was sand and the particular chelate used here has been reported to be fixed in soil in small amounts. The assumption that chelate molecules could be attached to the surface of the soil particles, even if it was sandy soil, lacks proof, but does not violate our limited knowledge of chelates.

GENERAL DISCUSSION AND CONCLUSIONS

Wallace (1955, 1956, 1960) and Hill-Cottingham (1957) believe that the entire chelate molecule, the chelating agent and the metal ion, is absorbed by the plant. Their proof is based on either the equal decrease in the chelating agent and the metal from the root medium, or detecting the chelating agent in the aerial part of the plant. Tiffin et al. (1960) and Brown et al. (1960) believe that the chelating agent acts just to make iron available to the root and very little emphasis can be placed on chelating agent absorption. Their evidence has been either in detecting an increase in the concentration of the chelating agent in the root medium which increased the chelating capacity of the nutrient solution, or in a small amount of the chelated iron in the plant, or a competition between the chelating agent and roots to retain iron.

In the work reported here, it has been shown that iron uptake in the non-chelate treatments was less than in the iron chelate treatments which was less than the hydrogen chelate treatments. Since the amount of iron supplied to all treatments was the same, it can be concluded that chelate has increased iron uptake. If the entire chelate molecule, the chelating agent and the iron molecule, was absorbed, it should have made iron content of iron chelate treatments higher than the hydrogen chelate treatments, which was not the case. By checking the analysis we can see that the iron concentration of iron chelate and hydrogen chelate treatments was 30 and 60 micrograms per gram higher than for the non-chelate treatment. With such results, where hydrogen chelate was twice as efficient as iron chelate in maintaining iron uptake, the idea of the entire molecule absorption could have no justification. It could be advocated that the chelating capacity of hydrogen chelate was partially exhausted by reaction with other cations in the medium, such as calcium, consequently decreasing the extent of competition between roots and the chelating agent to retain the iron molecule. Admitting such a competition exists between the roots and the chelating agent has the same meaning as admitting the absorption of iron molecule, without the chelating agent, because if the entire molecule was absorbed there would be no competition. It can be said that the efficiency of the hydrogen chelate in supplying iron was superior to iron chelate because in the former case plants had two sources of iron--that from the ionic form and that amount which reacted with the chelating agent to form iron chelate. But this statement ignores several facts. The total amount of iron supplied in each case was equal. Even assuming that the applied chelating agent would react with the entire amount of the iron applied, it could exceed the iron absorption of the non-chelate treatment by only the same magnitude as found for the iron chelate treatment. The difference, however, was almost twice as great. A third fact concerns calcium and potassium concentration and possibly other cations which were not investigated. There was a tendency, though very faint, for the plant to have the least concentration of these elements with the hydrogen chelate treatment. The conclusion is the same in this case, too; that is, if the entire chelate molecule was absorbed, it would be expected to yield higher concentrations of these cations associated with hydrogen chelate treatment, which was not the case.

If the chelate molecule is not absorbed by the plant, what effect might it have on phosphorus availability to the plant? Bear (1955) gave the impression that chelates increase phosphorus availability. Brown et al. (1960) reported some data in which phosphorus concentration in plant increased by increasing chelate concentration in the root medium. Perkins and Purvis (1954), with soil studies, found that treating the soil with EDTA increased the amount of the available phosphorus. Brown (1956) believes that EDTA can remove iron from ferric phosphate which increases phosphorus solubility. Bradley and Sieling (1953) found that several sugars and organic acids, which react with metal ions in a similar way to chelates, decrease the amount of precipitation of phosphate by iron and aluminum oxides. Struthers and Sieling (1950) found that phosphates precipitated by iron and aluminum could be rendered soluble by lowering the pH to a range of 4.0 to 6.0 when certain organic acids as tartrate, oxalate, and malate were present.

DeRemer (1959, 1961) postulated a theory which explains how chelates increase phosphorus availability to the plants. He states that

> there is a cycle, in which the chelating agents act as a carrier. The chelate introduced and the plant removes a cation from the chelate molecule. This leaves the complexing agent free to chelate another cation. If there is ferric phosphate present, the complexing agent can chelate some of the iron, leaving the phosphate in a soluble form.

DeRemer's cycle necessitates the presence of the phosphorus source, the chelate, and the plant, to obtain a positive effect of chelates on increasing phosphorus availability. This condition was met in the first experiment of this work, but not in the second experiment, though the response was observed in both cases. Several modifications are required to explain the mechanism by which chelates increase phosphorus availability. Olsen (1953) reported that it has been found that soluble salts decrease phosphorus solubility in soils. So any factors that decrease the concentration of the soluble salts, such as a chelating agent which complexes metal ions, would increase phosphorus availability. In the leaching experiment, there has been a decrease in calcium content of the leachate. If calcium is considered to be a representative of the cations in the medium, a condition more favorable to phosphorus solubility is created. However, the results of the same experiment showed an increase in ion concentration, indicated by more calcium and by a higher conductivity when the high concentration of chelate was used. This gives the impression that chelate increased phosphorus availability through another mechanism, such as decreasing the pH of the medium. So a chelating agent, either applied to the medium as such, or after the iron was removed by the roots from the iron chelate, reacts with metal ions to form the chelate structure and decreases the pH of the medium. This viewpoint is introduced by Martell and Calvin (1952) who reported that

> all metal chelates may be considered as formed by displacement of one or more usually weak acidic protons of the chelating agent by a metal ion. Thus, the addition of glycine to a solution of cupric salt causes a drop in the pH. The greater the tendency of metals to combine with a given chelating agent, the greater the drop in pH.

By decreasing the pH of the medium it is expected that conditions more favorable to phosphorus solubility are available.

The effect of decreasing the pH could serve as a basis to reconcile the two different direction effects of chelate, on increasing the solubility of salts. In the lower concentration of chelate, the change of pH is almost negligible, so the only active factor would be that tending to decrease the soluble cations from solution. In the higher concentration, the decrease in the pH is relatively effective, which resulted in higher concentration of ions in the solution. The amount of this increase has been greater than that amount retained by the chelate molecule, either as a metal ion to form the chelate structure or as cations retained by the mixed chelate molecule to balance the charge inequilibrium.

Any decrease in the pH, if it happened, could not be detected in the leachate. So, the significant effect of increasing phosphorus availability needs to be explained by another possibility. A concept of the mixed chelate formation provides this possibility. A mixed chelate is a chelate in which the active groups surrounding the metal ion in the chelate structure are not similar to each other. It has been mentioned by Martell and Calvin (1952) that phosphate could be one of the principal donor groups. Bogucki (1962) has proved the formation of mixed chelate for Th(IV) by bonding simultaneously 6 sexadenate ethylenediaminetetra acetic acid (EDTA) and bidenate 1, 2-dihydroxy-henzene-3, 5-disodium disulfonate (Tiron) in a 1:1:1 molar ratio. He also showed that Fe(III) forms a mixed chelate with quadridenate nitrilotriacetic acid (NTA) and bidenate Tiron. Thompson and Loraas (1962) also reported the formation of mixed chelate.

Now, to understand the mechanism by which chelates increase phosphorus availability, it could be imagined that upon iron chelate application to the medium it introduces the iron ion to the roots while the chelating agent remains in the medium. After the metal ion is extracted from the chelate, it is possible that it would lose the chelate structure and would be similar to a fresh chelating agent. This chelating agent would react with cations which are present in the medium such as iron or calcium or magnesium or whatever it were to form a new structure. Such reaction would decrease the soluble salts concentration as well as the pH of the medium, a result which encourages the solubility of the added phosphorus. Upon the formation of the new chelate structure, phosphate could be incorporated in the structure acting as an active group, and the mixed chelate is initiated. The mixed chelate, upon getting in touch with roots loses its metal ion, which is an essential factor in maintaining the chelate structure and the structure is disintegrated. Phosphorus content of the mixed chelate has a better chance to be absorbed by the roots while the chelating agent, beacuse it is not absorbed, is involved in initiating a new cycle.

SUMMARY

In order to evaluate the effect of chelate on the solubility, mobility, and availability of phosphorus, two experiments have been set up using superphosphate and rock phosphate as soluble and insoluble phosphorus sources. In the first experiment, Moscow tomatoes were grown by sand culture method in the greenhouse. Phosphorus was applied from two sources, either rock phosphate, with two levels, 66 and 132 pounds phosphorus per acre, or superphosphate with one level, 66 pounds phosphorus per acre. Iron was supplied either as iron chelate or iron citrate at a concentration of 1:13 ppm in both cases. Hydrogen chelate was applied to give the same chelate concentration as given by the iron chelate addition.

It was found that chelate increased phosphorus availability to the plant. This increase has been explained as a result of one or more of the following items: (1) DeRemer's cycle, (2) a drop in the pH, (3) the formation of mixed chelate, or (4) the reaction of the excess iron with phosphorus to form iron phosphate.

Iron absorption was decreased by increasing phosphorus levels which indicates iron precipitation in the root medium. Hydrogen chelate increased iron uptake more than iron chelate. The reason was given as one or a combination from the following items: (1) two sources of iron, (2) the competition between the roots and the chelating agent to retain iron ions was less, or (3) the formation of mixed chelate, which decreased iron availability in iron chelate.

The concentration of the nutrients in roots was related to the

corresponding concentration in the aerial parts to give an idea about the relative nutrient distribution throughout the plant. This ratio was called the translocation ratio.

The ratio indicated that phosphorus movement to the tops has been depressed by application of chelate because of the high iron uptake in this case. Chelate depressed iron movement, too. Phosphorus has improved the iron translocation ratio.

In all the reported work, the concentration of the nutrient in dry matter was used in drawing conclusions, but it should be added here that the conclusions would be the same if the total uptake, rather than the concentration, was used.

In the second experiment, cylindrical plastic tubes were filled with fine sand and amounts of superphosphate containing 14.5, 29, 44, and 88 pounds phosphorus per acre and were leached with water or hydrogen chelate or iron chelate at 2 ppm concentration. Additional chelate concentration of 6 ppm was used in the highest two levels of phosphorus. Rock phosphate was used as a phosphorus source with two levels, 44 and 88 pounds phosphorus per acre, leached with water and chelates in the low and high concentration. The leaching was done for four days; the leachate of every day was kept separate. In each leachate, conductivity, pH, iron chelate concentration, calcium content, and phosphorus content were determined. In the levels of 44 and 88 pounds of phosphorus of the superphosphate treatment, samples of sand were taken from the surface and depths of 2, 4, 8, and 12 inches to determine if there was any effect of the different leaching solutions on phosphorus mobility.

In this experiment it was found that chelate decreased ion concentration if low concentration of chelate was used while ions concentrations was increased if high concentration of chelate was used. This was explained to be a consequence of the different mechanisms by which chelate exerts its effect on phosphorus. In the low concentration the major effect of chelate is through decreasing ions concentration, mainly calcium, while in the high chelate concentration the major factor is the decrease in the pH of the medium which encourages phosphorus solubility. Mixed chelate formation takes place in each case.

The results of the two experiments were used to give a comprehensive explanation to the role of chelate in increasing phosphorus availability to the plant. The following equations diagram the conclusion:

1. Fe chelate + plant $\longrightarrow$ Fe plant + H chelate
2. H chelate + $(\text{cation})^+$ $\longrightarrow$ cation chelate

   pH of the medium decreases

   = more phosphorus solubility
3. Cation chelate + phosphate $\longrightarrow$ mixed chelate

   = more phosphorus availability.

The results of the plant growth experiment have referred to some possible relation between chelate and the early flower initiation. A work primarily set up to completely investigate this relation might increase our knowledge about chelate-plant relations. Also this experiment has referred to some effect of the interaction between chelate and phosphorus to have some effects on plant height. More work on this point is suggested.

Sand has been used in the leaching experiment as soil material.

It is reasonable to investigate the validity of the conclusions drawn from this experiment when ordinary soil, rather than sand, is used. The work concerned with phosphorus mobility in the soil profile should have the soil samples from much closer depths since phosphorus is known to be one of the most immobile elements in the soil.

Since chelate is known as a rather expensive material, the economical values of these results should be evaluated before they are suggested to the farmer.

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APPENDIX

Table 19. Analysis of variance of data for heights of plants on August 14, 1961, reported in table 2

Source of variation	df	Sum of squares	Mean squares
Replication	3	4.523	1.508
Leaching solution	2	12.122	6.061
Rep. x leaching solution	6	16.853	2.809
Phosphorus level	2	2.022	1.011
Leaching solution x P level	4	28.592	7.148**
Error	18	25.706	1.428
Total	35	89.820	

**Significant at .01 level.

Table 20. Analysis of variance of data for final heights of plants, reported in table 2

Source of variation	df	Sum of squares	Mean squares
Replication	3	40.159	13.386
Leaching solution	2	9.521	4.761
Rep. x leaching solution	6	12.669	2.112
Phosphorus level	2	1.875	0.938
Leaching solution x P level	4	18.480	4.620
Error	18	163.160	9.064
Total	35	245.864	

Table 21. Analysis of variance of data for weight of roots, reported in table 2

Source of variation	df	Sum of squares	Mean squares
Replication	3	3.742	1.248
Leaching solution	2	0.915	0.458
Rep. x leaching solution	6	16.698	2.783
Phosphorus level	2	2.282	1.141
Leaching solution x P level	4	16.329	4.082
Error	18	37.643	2.091
Total	35	77.610	

Table 22. Analysis of variance of data for weight of tops, reported in table 2

Source of variation	df	Sum of squares	Mean squares
Replication	3	22.602	7.534
Leaching solution	2	27.040	13.520
Rep. x leaching solution	6	148.973	24.829
Phosphorus level	2	138.627	69.313*
Leaching solution x P level	4	125.018	31.255
Error	18	238.344	13.241
Total	35	700.604	

*Significant at .05 level.

Table 23. Analysis of variance of data for total yield, reported in table 2

Source of variation	df	Sum of squares	Mean squares
Replication	3	30.89	10.30
Leaching solution	2	60.48	30.24
Rep. x leaching solution	6	231.80	38.63
Phosphorus level	2	122.12	61.06
Leaching solution x P level	4	204.49	51.12
Error	18	430.74	23.93
Total	35	1080.53	

Table 24. Analysis of variance of data for phosphorus concentration in roots, reported in table 3

Source of variation	df	Sum of squares	Mean squares
Replication	3	7020.80	2340.27
Leaching solution	2	23384.90	11692.45**
Rep. x leaching solution	6	3935.00	655.83
Phosphorus level	2	178161.90	89080.95**
Leaching solution x P level	4	16657.90	4164.48*
Error	18	15012.40	834.04
Total	35	244172.90	

*Significant at .05 level.
**Significant at .01 level.

Table 25. Analysis of variance of data for iron concentration in roots, reported in table 3

Source of variation	df	Sum of squares	Mean squares
Replication	3	21153	7051
Leaching solution	2	21903	10952
Rep. x leaching solution	6	24047	4008
Phosphorus level	2	14240	7120
Leaching solution x P level	4	1347	337
Error	18	77505	3195
Total	35	160196	

Table 26. Analysis of variance of data for calcium concentration in roots, reported in table 3

Source of variation	df	Sum of squares	Mean squares
Replication	3	0.422	0.141
Leaching solution	2	0.311	0.156
Rep. x leaching solution	6	1.261	0.210
Phosphorus level	2	0.878	0.439
Leaching solution x P level	4	4.269	1.067**
Error	18	2.328	0.129
Total	35	9.469	

**Significant at .01 level.

Table 27. Analysis of variance of data for potassium concentration in roots, reported in table 3

Source of variation	df	Sum of squares	Mean squares
Replication	3	1.113	0.371
Leaching solution	2	2.412	1.206
Rep. x leaching solution	6	3.015	0.503
Phosphorus level	2	4.330	2.165*
Leaching solution x P level	4	3.367	0.842
Error	18	7.332	0.407
Total	35	21.893	

*Significant at .05 level.

Table 28. Analysis of variance of data for phosphorus concentration in tops, reported in table 4

Source of variation	df	Sum of squares	Mean squares
Replication	3	1759.40	586.47
Leaching solution	2	554.00	277.00
Rep. x leaching solution	6	1398.00	233.00
Phosphorus level	2	64478.00	32239.00**
Leaching solution x P level	4	4043.00	1010.75
Error	18	9692.40	538.47
Total	35	81924.80	

**Significant at .01 level.

Table 29. Analysis of variance of data for iron concentration in tops, reported in table 4

Source of variation	df	Sum of squares	Mean squares
Replication	3	809	270
Leaching solution	2	4514	2257*
Rep. x leaching solution	6	2466	411
Phosphorus level	2	10447	5223**
Leaching solution x P level	4	1166	291
Error	18	9519	529
Total	35	28920	

*Significant at .05 level.
**Significant at .01 level.

Table 30. Analysis of variance of data for calcium concentration in tops, reported in table 4

Source of variation	df	Sum of squares	Mean squares
Replication	3	0.284	0.095
Leaching solution	2	0.082	0.041
Rep. x leaching solution	6	0.693	0.116
Phosphorus level	2	5.657	2.828**
Leaching solution x P level	4	0.359	0.090
Error	18	0.705	0.039
Total	35	7.779	

**Significant at .01 level.

Table 31. Analysis of variance of data for potassium concentration in tops, reported in table 4

Source of variation	df	Sum of squares	Mean squares
Replication	3	0.512	0.171
Leaching solution	2	0.653	0.326
Rep. x leaching solution	6	1.628	0.271
Phosphorus level	2	1.435	0.717
Leaching solution x P level	4	0.192	0.048
Error	18	2.583	0.144
Total	35	7.002	

Table 32. Analysis of variance of data of table 8

Source of variation	df	Sum of squares	Mean squares
Replication	1	2,430.00	2,430.00
Leaching solution	2	18,711.70	9,355.80
Rep. x leaching solution	2	13,115.00	6,557.00
Phosphorus level	4	630,911.70	157,727.90**
P level x leaching solution	8	31,088.30	3,886.00
Error a	12	38,080.00	3,173.34
Period	3	7,711,483.00	2,570,494.00**
Leaching solution x period	6	32,281.70	5,380.20
P level x period	12	1,750,141.70	145,845.20**
Leaching sol. x P level x period	24	95,318.30	3,957.90
Error b	45	127,875.00	2,841.66
Total	119	10,451,436.70	

**Significant at .01 level.

Table 33. Analysis of variance of data of table 9

Source of variation	df	Sum of squares	Mean squares
Replication	1	26.10	26.10
Leaching solution	2	3,648.50	1,924.20**
Rep. x leaching solution	2	64.90	32.40
Phosphorus level	4	51,095.00	12,774.00**
P level x leaching solution	8	1,007.20	124.20**
Error a	12	362.80	30.23
Period	3	315,293.50	105,098.00**
Leaching solution x period	6	5,242.70	873.80**
P level x period	12	136,818.90	11,401.50**
Leaching sol. x P level x period	24	2,902.90	121.00**
Error b	45	1,326.70	29.48
Total	119	517,788.70	

**Significant at .01 level.

Table 34. Analysis of variance of data of table 10

Source of variation	df	Sum of squares	Mean squares
Replication	1	5.8	5.8
Leaching solution	2	2.1	1.1
Rep. x leaching solution	2	18.1	9.1
Phosphorus level	4	62,301.7	13,075.4**
P level x leaching solution	8	212.6	26.6*
Error a	12	71.8	6.0
Period	3	238,827.9	79,607.3**
Leaching solution x period	6	12.6	2.1
P level x period	12	168,286.8	14,023.1**
Leaching sol. x P level x period	24	659.3	27.5*
Error b	45	284.9	6.3
Total	119	470,683.6	

*Significant at .05 level.
**Significant at .01 level.

Table 35. Analysis of variance of data of table 11

Source of variation	df	Sum of squares	Mean squares
Replication	1	67.5	67.5
Leaching solution	4	22,213.4	5,553.3**
Rep. x leaching solution	4	1,003.3	250.8
Phosphorus level	2	1,133,926.7	566,963.3**
P level x leaching solution	8	8,906.7	1,113.3*
Error a	10	2,816.7	281.7
Period	3	10,366,275.8	3,455,425.0**
Leaching solution x period	12	8,853.3	737.8**
P level x period	6	2,224,426.7	370,738.0**
Leaching sol. x P level x period	24	12,006.7	500.2**
Error b	45	6,862.5	152.5
Total	119	13,787,359.2	

*Significant at .05 level.
**Significant at .01 level.

Table 36. Analysis of variance of data of table 12

Source of variation	df	Sum of squares	Mean squares
Replication	1	35.2	35.2
Leaching solution	4	3,954.9	988.7**
Rep. x leaching solution	4	135.2	33.8
Phosphorus level	2	77,294.3	38,647.1**
P level x leaching solution	8	1,246.4	156.0*
Error a	10	346.7	34.7
Period	3	433,272.6	144,424.2**
Leaching solution x period	12	6,555.8	546.3**
P level x period	6	194,383.3	32,399.0**
Leaching sol. x P level x period	24	4,025.6	167.7**
Error b	45	1,835.4	40.8
Total	119	723,085.6	

*Significant at .05 level.
**Significant at .01 level.

Table 37. Analysis of variance of data of table 13

Source of variation	df	Sum of squares	Mean squares
Replication	1	75.7	75.7
Leaching solution	4	1,167.3	291.8**
Rep. x leaching solution	4	71.0	17.8
Phosphorus level	2	104,464.8	52,232.4**
P level x leaching solution	8	1,244.9	143.1
Error a	10	481.4	48.1
Period	3	423,198.8	141,066.3**
Leaching solution x period	12	3,185.1	265.4**
P level x period	6	279,872.7	47,312.1**
Leaching sol. x P level x period	24	3,514.7	146.4
Error b	45	4,520.9	100.5
Total	119	819,194.9	

**Significant at .01 level.

Table 38. Analysis of variance of data for phosphorus recovery as affected by the low concentration of chelate, reported in table 14

Source of variation	df	Sum of squares	Mean squares
Replication	1	176	176
Phosphorus level	3	28401	9467
Error a	3	8605	2268
Leaching solution	2	9104	4552
P level x leaching solution	6	31726	5287
Error b	8	23320	2915
Total	23	99534	

Table 39. Analysis of variance of data for phosphorus recovery as affected by the high concentration of chelate, reported in table 14

Source of variation	df	Sum of squares	Mean squares
Replication	1	110	110
Phosphorus level	1	1170	1170
Error a	1	781	781
Leaching solution	4	67889	16972*
P level x leaching solution	4	5066	1266
Error b	8	24836	3104
Total	19	99855	

*Significant at .05 level.

Table 40. Analysis of variance of data for phosphorus mobility, reported in table 15

Source of variation	df	Sum of squares	Mean squares
Replication	1	0.001	0.001
Phosphorus level	2	5.013	2.506**
Error a	2	0.060	0.030
Leaching solution	4	0.345	0.086*
P level x leaching solution	8	0.068	0.009
Error b	12	0.204	0.017
Depth	4	1.889	0.472**
P level x depth	8	0.528	0.066*
Leaching solution x depth	16	0.372	0.023
P level x leaching solution x depth	32	0.501	0.016
Error c	60	1.446	0.024
Total	149	10.426	

*Significant at .05 level.
**Significant at .01 level.

Table 41. Analysis of variance of data of table 16

Source of variation	df	Sum of squares	Mean squares
Replication	1	403.3	403.3
Leaching solution	4	9,055.0	2,264.0**
Rep. x leaching solution	4	505.0	126.2
Phosphorus level	2	3,821.7	1,910.9**
P level x leaching solution	8	2,945.0	368.1*
Error a	10	1,066.7	106.7
Period	3	1,963,620.0	65,454.0**
Leaching solution x period	12	13,805.0	1,150.0
P level x period	6	2,385.0	397.5
Leaching sol. x P level x period	24	6,215.0	259.0
Error b	45	42,724.8	949.4
Total	119	2,011,146.7	

*Significant at .05 level.
**Significant at .01 level.

Table 42. Analysis of variance of data of table 17

Source of variation	df	Sum of squares	Mean squares
Replication	1	0.004	0.004
Leaching solution	4	0.421	0.105**
Rep. x leaching solution	4	0.030	0.008
Phosphorus level	2	0.100	0.050**
P level x leaching solution	8	0.175	0.022**
Error a	10	0.032	0.003
Period	3	1.317	0.439**
Leaching solution x period	12	0.604	0.050**
P level x period	6	0.052	0.009
Leaching sol. x P level x period	24	0.267	0.011
Error b	45	0.509	0.011
Total	119	3.511	

**Significant at .01 level.

Table 43. Analysis of variance of data of table 18

Source of variation	df	Sum of squares	Mean squares
Replication	1	2.1	2.10
Leaching solution	4	611.2	152.80**
Rep. x leaching solution	4	11.8	2.96
Phosphorus level	2	110.1	55.00**
P level x leaching solution	8	54.6	6.83*
Error a	10	16.0	1.60
Period	3	3482.0	1160.30**
Leaching solution x period	12	185.3	15.40**
P level x period	6	81.4	13.60**
Leaching sol. x P level x period	24	83.3	3.50
Error b	45	171.4	3.81
Total	119	4727.9	

*Significant at .05 level.
**Significant at .01 level.