EXPERIMENTAL STUDIES ON SULFUR OXIDATION BY MICRO-ORGANISMS.

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PART ONE.

EXPERIMENTS ON THE COMPOSTING OF ROCK PHOSPHATE WITH SULFUR IN SLIGHT ALKALINE, CALCEREOUS SOILS.

INTRODUCTION.

The laboratory studies made by Lipman, McLean and Lint (10,11) on the effect of oxidation of sulfur in soils upon the solubility of rock phosphate laid to the positive conclusion that the method of composting rock phosphate with sulfur could be made practical and moreover would have an advantage over the old methods of making phosphoric acid rapidly available for plant food.

In continuing their investigations they have shown that mixtures in soil, rock phosphate and sulfur for plants grown in pot cultures compare favorably in value with commercial products.

The purpose of the work of Lipman and his assistants at the start was to produce a compost mixture which could easily be made by farmers. As soon as laboratory experiments are brought to the farm a number of difficulties arise, for example, in regard to such questions as mixing, the optimum water holding capacity of the compost and proportion of soil, to say nothing about storing, temperature and the length of time required. When such general laboratory experiments are being duplicated in the factory still more problems have to be solved, which do not concern the workers in the Agricultural Experimental Stations.

One of the main problems is the time required before the cultures begin to make the tri-calcium-phosphate available for plantfood. McLean (9) found in his best cultures that after 15 weeks small amounts of phosphorus became available. The recently reported experiment by Lipman and Joffe (12) of the New Jersey Agricultural Experimental Station, show, however, that under favorable conditions this time may be considerably reduced. Such favorable conditions, as an abundance of soil, high initial acidity of the soil used, excess of sulfur, and rather high temperature, cannot easily be duplicated on large scale productions.

Another problem which seems extremely interesting was whether the sulfur oxidizing organisms in compost mixtures of rockphosphate, sulfur and a calcereous soil could produce sufficient acid to dissolve the phosphates. Lindet and Bruno (8) discussing this problem in the academie d'Agriculture de France doubted this,for as they say'the acid formed would by preference attack the calcium carbonate and not the tri-calcium phosphate.' The opportunity 1) given to the writer to make a study of these and other problems in France where the greatest part of the soils are calcereous,could bring out the fact whether or not these scientists were right in their assumptions.

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1) The writer wishes to express here his thanks to Dr. J.G.Lipman for the opportunity to carry on these studies in France,to Dr.Andre Helbronner,formely chef de laboratoire in the Sorbonne University at Paris for his cordial incouragements throughuot the period of investigations,and to Monsieur J.Gavelle,Directeur de la laboratoire de recherches des Etablissements Kuhlmann a Paris for the help rendered in analyzing the numerous samples.

Purpose of the work.

The work undertaken dealt mainly with the problem of how to reduce the first part of the incubation period in soil-rockphosphate-sulfur mixtures before the phosphorus becomes available when a calcereous,slightly alkaline soil is used,and of reducing the amount of such a soil in the mixture to a point which would make the process practical in the factory.

A study was made of:

1. Influence of temperature on the activities of the sulfur oxidizing organisms.
2. Influence of light.
3. Partial sterilisation of the mixtures.
4. Effect of stimulants.
5. Initial reaction.
6. Aeration.
7. Replacement of soil by other substances as a source of Nitrogen.
8. Reduction of the proportion of sulfur in the mixtures.

Experimental work.

Methods.

The composts were made by thoroughly mixing air dry portions of soil, rock phosphate, sulfur, and other materials used, if not otherwise stated, and were then placed in tumblers covered with glassplates. Each compost was weighed and the waterholding capacity determined according to the Hilgard (6) method on portions of it. Samples were weighed out and the relative acidity, hydrogen-ion concentration and citrate soluble phosphoric acid determined. The mixtures were then inoculated with soil extract known to contain the sulfur oxidizing organisms. The compost mixtures were incubated at 30°C and placed at laboratory temperature. Daily records taken of the roomtemperature showed a fluctuation of the temperature between 10°C and 21°C, the average temperature being 17°C. The compost were kept at 60 per cent of the waterholding capacity throughout the period of incubation. The amount of water lost by evaporation, determined by placing the tumblers on the scale pan, was added once each week to the composts kept at roomtemperature and twice each week to the composts incubated at 30°C. Each time, after the water was added, the mixtures were removed from the tumblers and thoroughly mixed to provide for sufficient aeration, and to break up the small aggregates formed.

Ammonium citrate phosphoric acid was determined by sieving the air dry sample through a 50 mm sieve. Two grams of this material was finally ground in a cobalt mortar with a total amount of 80 c.c. ammonium citatre (400 grams per Liter), decanted into a 200 c.c. flask under repeated grinding. The remainder of the material in the mortar was washed into the flasks with distilled water. The stoppered flasks were shaken and left standing for 15 hours at roomtemperature. They were then placed for one hour on a water bath at 40°C, cooled, made to volume, shaken and filtered. An aliquot taken precipitated with $MgNH_4Cl$, and washed five times with ammonia water (4 per cent). The filter with contents was left standing for a few hours to dry, transferred to a platinum crusible, ignited (placed at a blow flame till weight was constant), cooled and weighed as $P_2O_7Mg_2$.

The relative acidity was determined by transferring an air dry weighed sample to 200 c.c. flasks with 160 c.c. of boiling water, thoroughly shaken every five minutes throughout a period of one half hour, the flasks with contents cooled, made to volume and shaken again. After settling an aliquot was drawn off, boiledto expell carbon di-oxid, cooled and titrated with 50 normal Sodium hydroxid. Phenolphtalein was used as indicator and the liquid titrated untill a faint pink colour remained. The results in the tables however are given as 0.5 normal Sodium hydroxid.

The water extracts for the determination of the Hydrogen ion concentration were prepared according to the method of Gillespie (5). The hydrogen-ion concentrations as expressed in pH values of the extract were determined by the colorimetric method as recommended by Clark and Lubs (3), using the thymol sulfon phthalein, tetra bromo phenol sulfon phthalein, ortho cresol sulfon phthalein, and the di bromo thymol phthalein series. Duplicate determinations of the hydrogen-ion concentrations, but not of relative acidity nor of the phosphoric acid were made, except when the results did not agree with the constructed curves for the relation between acidity and available phosphoric acid.

From time to time sulfate determinations were made to check up with the relative acidity.

Plan of Experiment.

Several sets of experiments were arranged in a similar way and according to the following plan:

The proportions of the mixtures were:

100 grams of soil
120 grams of sulfur
400 grams of rock phosphate.

The soils used were slightly alkaline, calcereous clayey silt loams;the water extract requiring of from 2 to 4 c.c. 50 norm hydrochloric acid per 100 grams to neutralize and having pH values of from 7.1 to 7.6. These soils were poor in organic matter;and their water holding capacity was but 24 and 26 per cent.

The flowers of sulfur were from the Italian mines.

The rock phosphate used was Tunisian rock containing 25.99 per cent of total phosphoric acid and 56.79 percent of total tri-calcium phosphate.

The water holding capacity of the soil-sulfur-rock phosphate mixtures was 20 and 22 per cent.

Experimental Results.

Series I. Influence of Light, Temperature and Peptone.

Until the experiments reported below were begun nothing was known about the influence of temperature on the production of available phosphoric acid. Since then Shedd (15) has reported greenhouse experiments on the availability of phosphoric acid in compost mixtures and he concludes from his study that high temperature exerts a decided influence on the sulfate production and the available phosphorus.

McLean states that when peptone was added to the mixtures "sulfer was largely transformed into sulfites". He laid emphasis on the fact that peat, manure and peptone should not be added to the mixtures for "a compost is more efficient in the absence of large amounts of organic materials". Brown and Gwinn (1) showed in their experiments that more available phosphoric acid is produced where manure is included in the compost. Ellett and Harris (4) in their recently publiced work come to the same conclusion. It should be mentioned here that McLean used soils with sufficient organic matter, especially where he used rich greenhouse soils.

In earlier work it was demonstrated by several investigators that various soil types are not alike in their ability to render rock phosphate soluble. At the suggestion of Dr. Lipman compost experiments were carried on in different localities with different soil types. Aside from the "power" of soils to produce sulfates, which according to the results obtained by Shedd(14), Ellitt and Harris (4), and Brown and Kellog (12) varies with the soil, the addition of peptone to a soil poor in nitrogenous material could therefore throw light upon the problem of whether or not nitrogen in sufficient quantities is necessary for the activities of the sulfer organisms.

The experimental results of this series consist of the citrate soluble phosphoric acid made available during an incubation time of 22 weeks, the relative acidity produced, and of the hydrogen-ion concentrations expressed in pH values of these mixtures at definite intervals during this period. In order to determine what the effect of light on the activities of the sulfur organisms might be two sets of quaduplicate tumblers were placed on a laboratory table and two similar sets were kept in a dark closet underneath the table. To duplicates of these sets was added peptone equivalent to 3 per cent of the soil in the mixtures for the purpose of studying the influence of nitrogenous matter. Still two other duplicate sets were placed in an incubator at 30°C of which two mixtures received in addition a similar proportion of peptone. The results otained are given in table I.

TABLE I

Influence of temperature, light and organic material on the production of acidity and available phosphoric acid, and the change of hydrogen ion concentration in compost mixture.

(Acidity is expressed in C.C. N/5 NaOH required to neutralise 100 grams air dry compost. Citrate soluble P_2O_5 in percent of total P_2O_5 in the mixture).

		After											
		0 weeks			3 weeks			6 weeks			9 weeks		
No	Treatment	Acidity	PH	Sol. P_2O_5	Acidity	PH	Sol. P_2O_5	Acidity	PH	Sol. P_2O_5	Acidity	PH	Sol. P_2O_5
1	In darkness Lab. Temp	Alk. .04	7.2	percent .00	Alk. .02	7.0	percent .00	.3	6.6	percent .00	.8	6.4	percent .00
2	In darkness Lab. Temp 3% peptone	Alk. .04	7.2	.00	Alk. .02	7.0	.00	.3	6.7	.00	5.1	6.1	.09
3	In light Lab. Temp.	Alk. .04	7.2	.00	.0	7.0	.00	.4	6.6	.00	.8	6.4	.00
4	In light Lab. Temp 3% peptone	Alk. .04	7.2	.00	.0	7.0	.00	.6	6.6	.00	5.7	6.0	.1
5	In darkness 30°C	Alk. .04	7.2	.00	3.1	6.4	.06	5.1	5.9	.19	52.2	5.2	.53
6	In darkness 30°C 3% peptone	Alk. .04	7.2	.00	5.4	6.0	.09	11.0	5.8	.28	126.9	3.4	7.2

TABLE 1 CONTINUED

		After								
		12 weeks			18 weeks			22 weeks		
No	Treatment	Acidity	PH	Sol. P_2O_5	Acidity	PH	Sol. P_2O_5	Acidity	PH	Sol P_2O_5
1	In darkness Lab. Temp.	4.9	6.1	percent .00	26.0	5.6	percent .10	73.3	4.8	percent .40
2	In darkness Lab. Temp 3% peptone	80.0	5.2	.60	208.0	3.0	7.92	220.7	3.0	9.3
3	In light Lab. Temp	1.9	6.	.00	8.6	5.9	.20	45.8	4.9	.31
4	In light Lab. Temp 3% peptone	67.1	5.2	.60	76.7	4.7	.84	198.2	3.1	8.7
5	In darkness 30°C	104.8	3.4	2.92	111.2	3.3	3.1	132.1	3.2	5.2
6	In darkness 30°C 3% peptone	204.4	3.0	9.11	231.2	2.9	14.0	270.0	3.0	18.9

The relative acidity, hydrogen-ion concentration and available phosphoric acid increased in all cases regularly, which when plotted formed similar curves. The quantities of acids or acid salts increased contineously, but the intensity of the acid as indicated by the pH values remained the same after a certain point was reached, the acid being neutralized by the tri-calcium phosphate. If more sulfur had been applied the strenght of the acid produced would presumeble have been greater, but since only a small theoretical excess of sulfur was given and the cultures were not carried on till greater amounts of tri-calcium phosphate were made available, the pH values remained at from 2.9 to 3.0. Determinations of hydrogen-ion concentrations made at the end of every week showed slight differences from week to week in pH values, namely of from 2.9 to 3.2.

The influence of temperature was very marked throughout the time of experimentation, the higher temperature causing a much higher relative acidity and concequently producing more available phosphoric acid. Even in the mixtures to which peptone was added the accumulation of soluble P_2O_5 was twice as great when the compost was incubated at 30°C (No6) as when they were incubated at roomtemperature (No 2)

It may be seen at once that the nitrogenous material introduced in the form of peptone in this calcereous soil, which was poor in nitrogen, had a still greater influence. C Cultere number 5 kept at 30°C had after 22 weeks but 5.2 per cent of the total P_2O_5 made citrate soluble and had accumulated an acidity aquivalent to 132,1 c.c. .5 normal NaOH per 100 grams mixture,while culture number 2 kept at roomtemperature and to which peptone was added showed 9.3 per cent of the total P_2O_5 to have made available, and an acidity aquivalent to 220.7 c.c . .5 normal NaOH. This explains at the same time why additions of manure in certain cases,as found by other investigators,render more phosphoric acid available.The sulfur oxidizing organisms need a certain quantity of nitrogen to carry on their activities. The source of the nitrogen does not necessarely have to be of a organic origin as is shown in another part of this study. When the amounts of nitrogen in the soil are sufficient additions of nitrogenous materials become detrimental as is pointed out by McLean.

From this series it is evedent that calcereous slight alkaline soil does not inhibit the activities of the sulfur oxidizing organisms nor the atack of the tri-calcium phosphate and transforming it into a soluble form as had been supposed by Lindet and Bruno. In all cultures there was an accumulation of sulfates as expressed in terms of acidity, even in yhe cultures kept in the light at laboratory temperature and without additions of peptone.

The influence of light was,although not very pronounced, noticeable. Diffuse light in factories might be somewhat detrimental,but under more favorable conditions than these cultures presumebly very little.

From a number of cultures curves were constucted to bring out the relation between the accumulation of acidity, change of hydrogen-ion concentration (pH values),and quantities of soluble phosphoric acid produced. These relations are shown graphically in figure I and II. The curves in fig.number I are constucted from mixtures incubated at room-temperature,and the curves of fig.number II for the cultures incubated at 30°C. These two sets of curves show claerly the influence of temperature upon the activities of the sulfur oxidizing organisms. Cultures incubated at room temperature accumulated acidity and concequently change the hydrogen-ion concentration very slowly during the first weeks,but cultures incubated at 30°C commence to change the reaction of the mixtures instantly at the beginning of the incubation period,the rate of accumulation of acidity decreasing in the same way when a certain point is reached both for the cultures incubated at room temperature and for those incubated at 30°C. That part of the curve represents the point at which most of the phosphorus becomes available. The set of curves of fig,II brings out that the amounts of free acid increased with the increase of the temperature.

Influence of temperature upon the change of Hydrogen-ion concentration, acidity accumulation, and available Phosphoric acid.

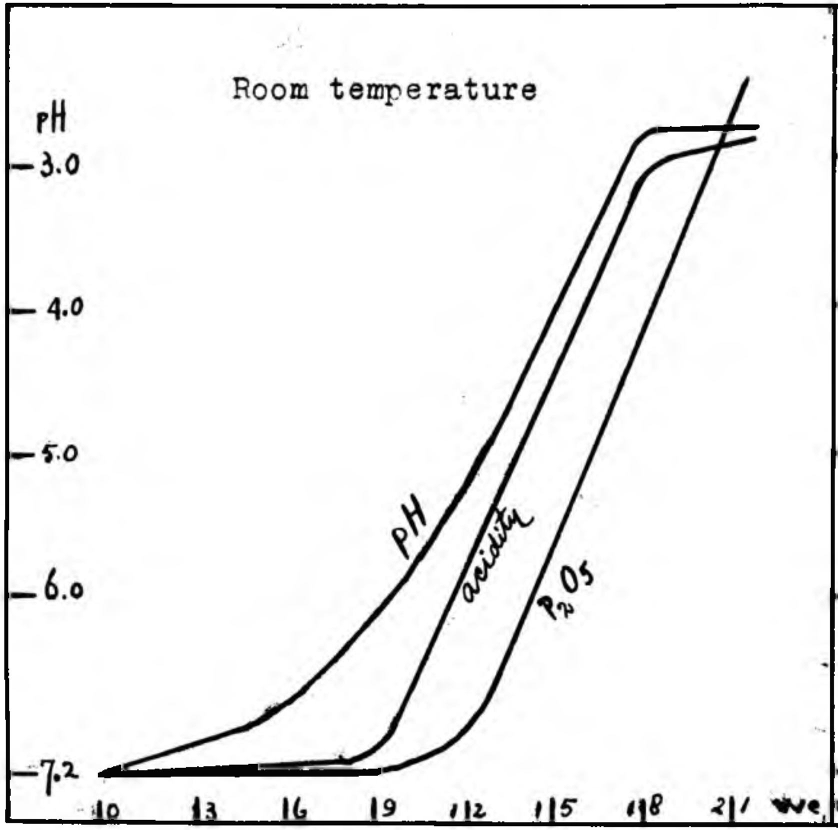

Fig.I

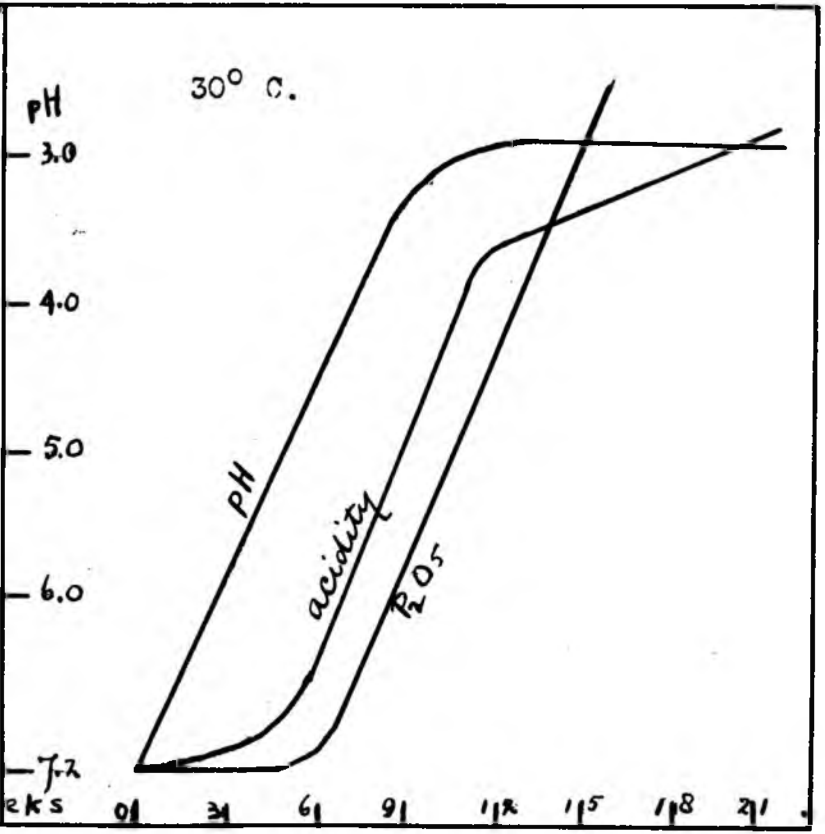

Fig.II

Series II. Stimulation.

In the work reported by McLean(9) a number of salts and organic materials were added to the ~~composts~~ mixtures in order to determine which salt or substance would exert a stimulating action on the sulfuroxidation process. He found that all salts used, except under certain conditions ferrous sulfate and aluminum sulfate, and a combination of the two salts, had no influence on the production of the available phosphoric acid. Since sulfuric acid and (or) acid sulfates are produced by the organisms it was thought that small amounts of H_2SO_4 added at the beginning would possibly stimulate their activities.

The soil used in this series consisted of an equal mixture of two different fine calcereous silt loams, which were slightly alkaline, their pH values being 7.4 and 7.5.

In one series the sulfuric acid was added before the mixtures were inoculated with the soil infusion and in another series immediately afterwards. The two series checked verey closely and the results obtained from the series to which the acid was added before the infusion was made are given in table 2, together with the treatment per 100 grams mixture. At the end of the first week peptone was added to all series aquivalent to 3 per cent of the soil used. All mixtures were incubated at roomtemperature. Determinations of acidity, hydrogen-ion concentrations and citrate soluble P_2O_5 were made at the end of every three weeks, but only the data obtained at the beginning, at the end of 9 weeks and at the end of 18 weeks are included as representatives of this series.

TABLE 2

Influence of Sulfuric acid in compost mixtures when a fine calcereous alkaline soil is used.

Treatment		After								
		0 weeks			12 weeks			18 weeks		
No	C.C. Norm. H_2SO_4 per 100 grams mixture	Acidity	PH	Sol. P_2O_5 %	Acidity	PH	Sol. P_2O_5 %	Acidity	PH	Sol. P_2O_5 %
1	1.8	Alk. .05	7.1	.00	79.8	4.5	2.36	146.4	3.6	9.65
2	3.8	Alk. .04	7.1	.00	77.4	4.6	2.28	165.5	3.5	10.40
3	5.8	Alk. .06	7.1	.00	77.8	4.6	2.30	197.3	3.4	12.10
4	6.8	Alk. .01	7.0	.00	78.5	4.5	2.20	160.2	3.2	11.4
5	7.8	Alk. .05	6.9	.10	73.7	4.6	2.08	126.1	3.5	9.15
6	9.8	Alk. .02	7.0	.08	73.3	4.6	2.12	90.6	3.6	8.8
7	11.8	Alk. .01	6.8	.93	67.7	4.7	1.79	87.4	3.6	7.17
8	13.8	Alk. .03	7.1	1.13	65.0	4.7	1.66	78.4	3.6	4.65
9	None	Alk. .04	7.2	.00	61.0	4.8	1.60	147.8	3.3	9.98

During the first eleven weeks culture numbers 1 to 7 particularly seemed more or less stimulated by the treatment as compared with the check cultures, but they gradually over-taken by the checks in acidity accumulation, till after 18 weeks several of the cultures were behind the checks. Culture numbers 2,3 and 4 however had at that time still a higher degree of acidity and also more available phosphoric acid than had the check cultures. From these and other experiments the conclusion can be drawn that when a fine calcereous silt loam is used, H_2SO_4 exerts a stimulating influence during the first part of the incubation period if quatities of approximately 7 to 10 liters (52° Be) per ton are used, but depresses the activities of the sulfur oxidizing organisms when larger amounts are added.

Several salts were tried out in the earilier work by McLean as possible catalytic agents. Some organic acids and two different acid salts which suggested themselves for several reasons had not been employed. As organic acid was for our experiment chosen acetic acid, which could possibly lower the alkalinity of the mixtures, and as acid salts sodium bi-carbonate as a possible source of carbon di-oxide and sodium bi-sulfite.

These constituents were added in small quantities to mixtures as previously described, but no additions of sulfuric acid were made. Treatment of the mixtures and the results obtained after 6 weeks and 12 weeks are reported in table3.

TABLE 3

Influence of Stimulation in composts
kept at room temperature.

		After								
		0 weeks			6 weeks			12 weeks		
No	Additions	Acidity	PH	Sol. P_2O_5 %	Acid ity	PH	Sol. P_2O_5 %	Acidity	PH	Sol. P_2O_5 %
1	None	Alc. .04	7.2	.00	5.2	5.5.	.09	103.6	3.3	6.69
2	2 cc Norm.CH_3COOH	Alc. .02	7.1	.00	.9	5.9	.00	71.4	4.8	.83
3	6 cc Norm.CH_3COOH	Alc. .02	7.0	.00	.4	5.9	.00	75.8	4.1	6.34
4	10 cc Norm.CH_3COOH	Alc. .01	7.0	.00	.4	6.0	.00	73.0	4.1	4.32
5	.01 percent $NaHCO_3$	Alc. .06	7.2	.00	27.5	5.5	(1)	187.9	4.8	13.94
6	.02 percent Na HCO_3	Alc. .05	7.2	.00	24.7	5.5		187.9	4.7	11.43

(1) Not determined.

Table 3 CONTINUED

		After								
		0 weeks			6 weeks			12 weeks		
No.	Additions	Acidity	PH	Sol. P_2O_5 %	Acidity	PH	Sol. P_2O_5 %	Acidity	PH	Sol. P_2O_5 %
7	.03 percent $NaHCO_3$	Alc. .04	7.2	.00	10.8	5.9	(1)	183.4	4.7	12.05
8	.1 percent $NaHSO_3$ (40%)	Alc. .4	7.3	.00	10.4	5.9		81.1	5.0	5.10
9	.2 percent $NaHSO_3$ (40%)	Alc. .4	7.3	.00	9.8	6.0		82.4	5.0	6.01
10	.3 percent $NaHSO_3$ (40%)	Alc. .4	7.3	.00	9.9	6.0		84.1	5.2	4.70
11	.5 percent Na HSO_3 (40%)	Alc. .5	7.3	.00	9.9	6.0		70.3	5.3	4.52
12	None	Alc. .04	7.1	.00	5.4	5.5	.00	104.4	3.4	7.1

(1) not determined.

Acidity and hydrogen-ion concentrations were determined at the end of every two weeks, but are not reported here since these data were merely used in checking up the procedure of the process of sulfate production.

The cultures to which acetic acid was added ran in duplicate very inconsistantly, but no stimulation could be noticed.

There was a gradual rise in acidity and consequently a gradual lowering of pH values in all mixtures, but although in culture numbers 5,6,and7, to which sodium bi-carbonate was added, a high relative aciditywas reached, the intensity of the acid produced as indicated by the low hydrogen-ion concentration (high pH values) seemed less. Nevertheless, a marked stimulating effect was to be noticed throughout the entire period of the experiment on the production of acidity and citrate soluble phosphoric acid. This might have been due to a production of CO_2 (probably needed by the sulfer organisms or for greater activity) when other bacteria were depressed to a high degree or stopped work in these acid media.

The cultures to which sodium bi-sulfite in solution was added as well as the cultures with acetic acid were behind the checks. The influence of $NaHSO_3$ was depressing rather than stimulating for these soil types.

Two series of cultures made up of the same constituents as described under series I, and placed at roomtemperature, one series in light and another in darkness, and two other series at 30° C which were similar treated. One tumbler in each series received a similar treatment with sulfuric acid as is given in table 2 and in addition a mixture of 0.02 per cent $FeSO_4$ and 0.02 per cent $Al_2(SO_4)_3$. The other tumblers of each series received the iron and aluminum salts but no sulfuric acid. The cultures with the addition of sulfuric acid and these two salts failed to show an increase above their respective checks of sulfates or soluble phosphoric acid and are therefore not included in the tables. The resutsof the series to which iron and aluminun were added but without sulfuric acid are given in table 4.

TABLE 4.

Influence of a mixture of .02 Ferrous sulfate and .02 Aluminum sulfate on the production of acidity and citrate soluble P_2O_5 in composts whereby a fine calcercous alkaline soil is used.

No	Treatment	After								
		0 weeks			6 weeks			12 weeks		
		Acid-ity	PH	Sol. P_2O_5	Acid-ity	PH	Sol. P_2O_5	Acid-ity	PH	Sol. P_2O_5
1	In darkness Lab.Temp.	Alk. .04	7.2	percent .00	.7	6.5	percent .00	5.3	6.0	percent .00
2	In darkness Lab. Temp and 3% peptone	Alk. .04	7.2	.00	5.1	6.1	.00	80.9	5.2	trace
3	In light Lab. Temp	Alk. .04	7.2	.00	.5	6.6	.00	3.6	--	.00
4	In light Lab. Temp. and 3% peptone	Alk. .04	7.2	.00	5.2	6.0	.00	78.7	5.2	trace
5	In darkness 30°C	Alk. .04	7.2	.00	5.0	6.1	.00	105.2	3.4	2.22
6	In darkness 30°C 3% peptone	Alk. .04	7.2	.00	9.3	5.9	.92	199.1	3.0	12.2

TABLE 4 CONTINUED.

		After					
		18 weeks			22 weeks		
No	Treatment	Acidity	PH	Sol. P_2O_5	Acidity	PH	Sol. P_2O_5
				percent			percent
1	In darkness Lab. Temp	20.7	5.4	.28	70.3	3.7	
2	In darkness Lab. Temp. and 3% peptone	209.0	3.0	12.65	217.2	3.0	(1)
3	In light Lab. Temp.	26.6	5.1	--	75.8	3.7	
4	In light Lab. Temp. and 3% peptone	89.8	3.5	1.12	183.9	3.1	
5	In darkness 30°C	107.2	3.3	2.71	132.6	3.2	
6	In darkness 30°C 3% peptone	216.7	3.0	14.07	250.0	3.0	

(1) not determined.

It is evident from these data that if stimulation occured it was by no means marked. This can partcularly be seen when table 4is compared with the results reported in table 1. The conclusion drawn from series I for the influence of temperature,light,and peptone hold also for this series.

SeriesIII. Partial Sterilisation.

It had been shown in the experiments of Lipman,McLean and Lint(11) that sterilisation of the mixtures was detrimental to the production of available phosphoric acid.No attempt was made to study the influence of partial sterilisation by means of salts or acids. The investigations of Russel (13) with soils treated with antiseptics showed an increase in bacterial numbers as the result of improvement of the soil medium. This worker used carbon di-sulfide and came to the conclusion that although thebacterial numbers were increased partial sterilisation did not inprove the bacterial flora. This was shown by the fact that the old flora,when reintroduced into partial sterilized soil,attained higher numbers and effected more decompositoin than the new flora. Partially sterilised soil plus 0.5 per cent of untreated soil,or an unfiltered aqueous extract of untreated soil,soon contained higher bacterial numbers per gram and accumulated ammomia at a faster rate than partially sterilised soil.alone.Truffaut (16) concluded after repeated trials that calcium sulphide was an exceptionally good mens of partial sterilisation. Hutchinson (7) studied the effect of caustic lime for partial sterilisation as apractical means and obtained good resuts. Many other investigators worked with different kinds of anticeptics like phenol, naphtaline, toluene, etc., which seemed less suitable for the purpose. The purpose of partial sterilisation in the case of the studies at hand would be to suppress undesirable bacterial growth in the mixtures and thereby favoring the development and perhaps multiplication of the sulfer oxidizing organisms.

On account of the relative high calcium content of the soils used and the abundance of calcium in the tri-calcium phosphate used, and in view of the necessary production of acidity caustic lime was not used. The exellent results obtained by Truffaut in the treatment of soil with CaS as a meansof partial sterilisation led the writer to conduct a series of experiments in which the cultures received different amounts of calcium sulphide. In thes preliminary studies it was brought out taht pure CaS and the technical product CaS in H_2SO_4 did not produce satisfactory results. The acidity obtained in compost mixtures after six weeks of incubation with a neutral rich garden soil (incubated at 28° C) was:

Nothing	added	98.4 cc. 5/N NaOH per 100 grams mixture,
CaS	,,	Alkaline
CaS- H_2SO_4	,,	12.1 cc. 5/N NaOH per 100 grams mixture.

For the partial sterilisation studies the same soil mixtures and the same proportion of sulfur and rock-phosphate were used as in series II. The compost mixtures were kept at room-temperature throughout the incubation preiod. The results obtained with 0.01,0.02 and 0.03 per cent of sodium chloride and a mixture of 0.01 per cent NaCl and 9 cc. 10/N hydrochloric acid per 100 grams mixture are recorded in table 5.

TABLE 5

Influence of partial sterilization on the production of acidity and available P_2O_5 in compost mixtures kept at room temperature.

		After											
	Treatment	0 weeks			3 weeks		6 weeks		10 weeks		13 weeks		
No	percent NaCl added	Acid-ity	PH	% sol. P_2O_5	Acid-ity	PH	Acid ity	PH	Acid-ity	PH	Acid ity	PH	% sol. P_2O_5
1	.01	Alk .04	7.2	.00	.09	7.0	.6	5.0	18.0	5.5	65.6	3.9	1.82
2	.02	Alk. .04	7.2	.00	.0	6.9	.0	6.9	20.5	5.4	57.2	4.9	trace
3	.03	Alk. .04	7.2	.00	Alk. .1	7.0	.0	6.9	22.8	5.3	55.7	4.9	.83
4	.01 -- 9 CC $\frac{10}{N}$ Hcl	Alk. .08	7.3	.00	Alk. .1	7.0	.5	5.9	20.5	5.4	55.7	4.9	trace
5	Check	Alk. .04	7.2	.00	.2	6.9	1.6	5.8	33.0	4.9	79.0	3.4	4.15

From these data it is readily to be seen that NaCl added in small quantities depressed considerably the growth and activities of the sulfur oxidizing organisms. Even where but 0.01 per cent NaCl was added the production of relative acidity after 13 weeks was less than in the check cultures and the available phosphoric acid was less than half the amount produced if compared with the cultures which received no additions of NaCl. Incidentally it was shown that soils secured near the sea coast and containing small amounts of chlorides were not suitable for rapid production of acidity and available P_2O_5. Bacterial counts showed that althoughfewer species of soil bacteria were present and smaller numbers existed,the sulfur oxidizing organisms were not favored by their absence. For the oxidation of sulfur in alkaline soils from California containing greater or less amounts of chlorides,the reader is referred to another part of the work.

The same soil-sulfur-rock-phosphate mixtures were used for cultures to which 10/N HCL was added. Additions varied from 4.5 cc. to 88 cc. per 100 grams mixture. The compost mixtures were incubated for the first six weeks at 30° C and than placed in a dark cubboard at roomtemperature. Water was added as usual and the mixtures stirred at the end of each week. Relative acidity and pH values were determined every time when stirred. Most of the free acid had been neutralized by the tri-calcium phosphate, but according to the low pH values considerable free acid was still present, at the start of the experiment, especially in culture numbers 9 and 10 as recorded in table 6.

Compost mixtures with additions of varying amounts of 10/N HCl per 100 grams mixture for partial sterilization. All composts incubated at 30°C during the first 6 weeks and thereafter at room temperature.

		After														
	Treatment	0 weeks			3 weeks			6 weeks			10 weeks			14 weeks		
No.	ccHCl added	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5
1	4.5	.04	6.8	.00	.01	6.8	.00	.4	6.6	.00	57.4	4.9	(1)	66.9	4.9	2.36
2	9	.1	6.6	.00	.0	6.9	.00	1.7	6.1	.00	67.8	4.7		68.4	4.8	2.13
3	14	.1	6.6	.00	.0	6.9	.00	1.6	6.1	.00	67.8	4.7		68.0	4.8	.77
4	18	.16	6.7	.00	.0	6.9	.00	.8	6.8	.00	56.0	4.9		64.4	4.8	.76
5	28	.12	6.7	.00	.0	6.9	.00	.4	6.8	.00	lost			lost		
6	38	.15	6.7	.00	.6	6.8	.00	1.2	6.2	.00	26.2	5.8		27.2	5.6	6.1
7	48	.16	6.7	.00	.0	7.0	.00	.4	6.7	.00	21.1	5.8		20.9	5.8	.68

(1) not determined.

Table 6 CONTINUED

		After														
	Treatment	0 weeks			3 weeks			6 weeks			10 weeks			14 weeks		
No.	ccHCl added	Acidity	PH	% Sol P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5
8	58	.5	6.2	.00	Alc. .05	7.1	.00	2.1	5.8	.00	8.3	6.0	(1)	10.1	5.8	.46
9	68	6.3	5.3	.68	.0	6.8	.66	.7	6.7	.22	3.2	6.6		5.3	6.4	0.19
10	88	7.7	5.2	.40	.0	6.8	.34	.7	6.7	.00	3.6	6.6		5.5	6.4	.00
11	Check	Alc. .04	7.3	.00	.2	6.6	.00	4.6	6.1	.00	55.4	5.0		104.2	3.2	5.2

(1) not determined.

After one week of incubation the pH values had gone up and approached the neutral point. There was but slight change at the end of the third week and culture number 8 was still slightly alkaline, indicated both by the pH values and the relative acidity at that time. At the end of 14 weeks all cultures to which HCl was added were far behind the check cultures, as well in relative acidity as in available P_2O_5. Bacterial activities has been very slight in culture numbers 9 and 10, and the small amounts of P_2O_5 available at the beginning of the experiment were transformed into insoluble phosphates. Even where but 4.5cc. 10/N HCl per 100 grams mixture was added it proved strongly detremental. The results obtained confirmed the conclusions drawn from the previous experiment that chlorides are detremental to the activities of the sulfur oxidizing organisms.

Series IV. Replacement of soil by other substances as a source of Nitrogen.

In his studies McLean (9) came to the conclusion that 100 parts of soil, 120 parts of sulfur, and 400 parts of rock phosphate would be the most economical combination for the production of available phosphoric acid. All investigators used great quantities of soil, based on the work of McLean. Some of them increased the amounts of soil or added manure to the origional proportions of soil-sulfur-rock-phosphate.

No attempt had been made to reduce the quantities of soil and replacing it by other material as sources of nitrogen except in the studies of pure cultures which were made in the laborarory of the New Jersey Agricultural Experiment Station, which studies were made mainly during the absence of the writer.

For practical purposes a great bulk of soil in the mixtures is undesirable. The high cost of handling and transportation are regarded as considerable items, aside from the problems of mixing and storing.

A series of experiments was conducted with a rather rich, slightly alkaline, calcereous garden soil. All mixtures were placed in a incubator at 30°C.

Influence of varying amounts of soil on the production of acidity
and soluble phosphoric acid . (Incubated at 30°).

		After								
		0 weeks			6 weeks			8 weeks		
No.	Compost mixture	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5
1	25 gr. soil 120 gr. sulfur,400 gr.rock phosphate	Alk. .08	7.2	.98	70.3	4.0	3.51	80.4	3.4	4.64
2	50 gr.soil, 120 gr. sulfur, 400 gr. rock phosphate.	Alk. .08	7.2	1.00	70.9	3.8	4.20	76.9	3.0	5.32
3	75 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	Alk. .08	7.3	.97	76.0	3.7	4.60	166.2	2.9	8.72
4	80 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	Alk. .06	7.1	.96	121.4	5 weeks 3.0	6.32	177.9	7 weeks 2.9	10.32
5	90 gr.soil, 120 gr. sulfur 400 gr. rock phosphate	Alk. .07	7.3	.90	86.8	3.6	5.60	172.3	2.9	10.00
6	100 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	Alk. .08	7.2	.92	67.6	4.1	2.80	131.0	2.9	7.32

Table 7 CONTINUED

		After								
		10 weeks			12 weeks			15 weeks		
No.	Compost mixture	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5
1	25 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	80.4	3.4	4.64	143.0	2.9	8.51	166.6	2.9	9.12
2	50 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	76.9	3.0	5.32	158.0	2.9	8.62	193.6	2.9	12.90
3	75 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	166.2	2.9	8.72	182.0	2.8	11.21	199.3	2.9	13.82
			11 weeks							
4	80 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	227.7	2.9	14.10						
5	90 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	232.7	2.9	14.00						
6	100 gr. soil, 120 gr. sulfur, 400 gr. rock phosphate	216.6	2.9	13.92						

Table 7 includes a part of the data secured, showing that cultures whith from 80 to 100 parts of this soil and respectivily 120 parts of sulfur and 400 parts of rock phosphate behaved very much the same. After 7 weeks two of these cultures had made more P_2O_5 available than the cultures with but 25 parts of soil had made available after 15 weeks, while culture numbers 4,5 and 6 had as much available phosphoric acid after 11 weeks as culture numbers 1,2 and 3 after 15 weeks. The difference between numbers 4,5 and 6 after 11 weeks both in relative acidity and available phosphoric acid was very small. It seemed therefore, that 80 parts of this soil was sufficient from which to expect good results.

From preliminary experiments conducted during this time at the Pasteur Institute and from previous experiments with peptone the conclusion was drawn that the activities of the sulfur oxidizing organisms depended largely on sufficient available nitrogen. During the progress ofthe work a more concentrated culture of strong sulfur oxidizing organisms for inoculation had been secured and was used in the experiments recorded in table 8.

TABLE 8

Influence of Ammonium sulfate when the soil is reduced to 10 parts, in a mixture of 100 parts of sulfur and 400 parts of rock phosphate.

		After											
		0 weeks			4 weeks			8 weeks			11 weeks		
No.	Additions	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5
1	None- Incubated at $30^\circ C$	.4	6.8	.00	130.5	3.5	4.60	195.7	2.9	.11.73	227.1	3.0	18.7
2	.2% $(NH_4)_2SO_4$ added at begin. Incubated at $30^\circ C$	.5	6.8	.00	114.6	3.6	3.82	194.2	3.0	10.00	243.7	3.0	22.3
3	.1% $(NH_4)_2SO_4$ added at begin. and 1% $(NH_4)_2SO_4$ added by stirring, at 30°	.5	6.8	.00	117.0	3.6	3.86	202.0	3.0	8.64	241.3	3.0	21.9
4	None, room temperature	.4	6.8	.00	67.4	4.1	2.18	151.4	3.2	7.95	175.7	3.0	16.86
5	.2% $(NH_4)_2SO_4$ added at begin. Room temperature	.5	6.8	.00	53.2	4.1	2.00	150.3	3.2	7.42	172.9	3.0	16.18
6	.1% $(NH_4)_2SO_4$ added to begin and .1% $(NH_4)_2SO_4$ added by stirring. Room temperature.	.5	6.8	.00	54.1	4.1	2.84	162.8	3.3	8.32	188.1	3.0	16.98

A mixture of 20 parts of soil,100 parts of sulfur and 400 parts of rock phosphate was used. It had previously been found that the quantity of sulfer could be reduced.to 100 parts,see the work reported in series V.
The 10 grams of soil were of the same lot used in the experiments reported in the previous table. In addition, ammonium sulfate aquivalent to 0.2 per cent of the mixture, was given to the cultures 2 and 4 at the beginning, and ammonium sufate aquivalent to 0.1 per cent of the mixtures to cultures 3 and 6,while another 0.1 per cent (in total) was added to cultures 3 and 6 stirred into during the first 8 weeks.

The available figures show that the compost mixtures to which ammonium sulfate was added and which were incubated at 30°C made 3.6 and 3.2 per cent more phosphoric acid available after 11 weeks than the ones with no additions of ammonium sulfate. The mixtures placed at room temperature however, were all practically alike after this incubation period. It seemed therefore,that enough nitrogenous matter was available up to a certain point. From then on the nitrogen source in the form of ammonium sulfate keeps the organisms supplied with sufficient nitrogen to carry on their activities. From other experiments (some of which are reported in tablt 11) it is known that if the quantities of a calcereous soil is reduced to 10 parts or less, sulfur oxidition by the organisms is very slow if not inhibited. It is interesting to note that in the case of these mixtures the temperature influence was far less pronounced than in the experiments reported in a earlierseries. Unfortunately, time was pressing and the cultures under discussion must be left behind. The data obtained later are not available, but cultures which had been allowed to go for 16 weeks showed at the end of this period 27.6 per cent available P_2O_5 without ammonium sulfate and 34.1 per cent available P_2O_5 with ammonium sulfate added. A part of this relative rapidity of oxidation was attributed to the fact that the small amounts of soil and the addition of ammonium sulfate resulted in a slightly acid medium.at the start of the experiments.

SERIES V. REDUCTION OF SULFUR.

In the commercial methods of making acid phosphate the proportions of sulfuric acid (52^{o}Be) and rock phosphate used are approximately 1:1. The amounts of sulfur employed in composting experiments have usually been larger than the amounts of sulfur used in the commercial methods. If smaller quantities were employed the available phosphoric acid decreased, From a commercial point of view it is interesting to approach the same approximate figures as used in the ordinary methods, namely 20 to 222 per cent of sulfur, especially where the cost of sulfur is high. An excess of sulfur would inhibit composting as might be done in countries where sulfur is redily to be had at a comparativily low price. Experiments were made with a soil rock phosphate mixture in which the amounts of sulfur were reduced to 50 per cent of the original amounts used by Mc Lean and which was pronounced by him as the most economical for farmers. The soil used was a slightly alkaline garden soil containing apperently sufficient nitrogen for the sulfur oxidizing organisms to produce good results. The compost mixtures were incubated at 30° C and a part of the results obtained are given in table 9.

TABLE

Influence of reduction of sulfur on the production of acidity and available P_2O_5 . (Incubated at 30°C) .

No		0 weeks			12 weeks			18 weeks		
		Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5
1	120 gr. sulfur	.4	6.9	.00	210.9	3.0	11.44	266.0	2.9	25.0
2	110 gr. sulfur	.4	6.9	.00	212.8	3.0	10.00	247.0	3.0	24.4
3	100 gr. sulfur	.4	6.9	.00	198.1	3.1	10.00	247.0	3.0	24.2
4	90 gr. sulfur	.4	6.9	.00	203.5	3.0	9.74	246.0	3.0	24.0
5	80 gr. sulfur	.4	6.9	.00	186.9	3.1	9.92	213.0	3.0	18.2
6	70 gr. sulfur	.4	6.9	.00	182.0	3.1	8.1	204.2	3.0	16.1
7	60 gr. sulfur	.4	6.9	.00	193.0	3.1	8.3	201.0	3.1	13.2

From the reported data it is obvious that less sulfur than the theorectical amounts necessary to transform the total phosphoric acid in a soluble form are impractical. It may be seen from the data in this table that a quantity approaching the theoretical amount is sufficient to warrant goog results. One hundred grams of sulfur, 100 grams of soil and 400 grams of rockphosphate gave as good results as 110 grams of sulfur with the same proportional amounts of soil and rock phosphate, and nearly as good as when 120 grams of sulfur was employed. When the sulfur was reduced too far the available phosphorie acid decreased accordingly.

SERIES VI. INITIAL REACTION.

TO make a fair test as to whether or not initial reaction would have any influence, 100 parts of an alkaline garden soil, 100 parts of sulfur and 400 parts of rock phosphate were composted and treated at the beginning of the experiment, with different amounts of sulfurous acid in some cases, and in other cases by adding sulfurous to the mixtures when they were stirred for aeration, From the preliminary experiments it was concluded, that approximately 13 cc. sulfurous acid of a strenght neutralizing the same volume of normal sodium hydroxid added per 100 gram mixture was best suited for the purpose. The mixtures were incubated at room temperature and at 30°C, and the data secured are reported in table 10.

TABLE 10
Influence of initial reaction, using sulfurous acid, on the production of acidity and available phosphoric acid.

		After											
		0 weeks			6 weeks			12 weeks			15 weeks		
No	Treatment	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5
1	None (30°C)	Alk. .4	7.3	.00	28.7	5.5	--	198.1	3.0	10.00	211.4	3.0	12.66
2	13 cc SO_2 in H_2O per 100 grams mixture added by stirring (30°C)	.4	6.6	.00	38.8	5.4	--	157.5	3.4	6.74	231.8	3.0	14.12
3	13 cc SO_2 in H_2O added at begin. (30°C)	1.4	6.0	trace	98.6	3.2	3.7	212.6	2.8	13.63	247.6	3.1	17.94
4	None (room temperature.)	Alk. .4	7.3	.00	3.4	5.6	--	31.1	5.5	.71	91.2	3.4	3.08
5	13 cc SO_2 in H_2O added by stirring (room temperature)	.4	6.6	.00	21.9	5.5	--	22.8	5.6	.45	194.7	3.1	11.89
6	13 cc SO_2 in H_2O added at begin. (Room temperature)	1.4	5.9	trace	36.3	5.5	--	181.8	3.2	7.95	218.5	3.0	15.38

The influence of sulfurous acid on the mixtures is very obvious. Although the influence after 15 weeks on the mixturesincubated at 30°C is not so pronounced as on the mixtures incubated at room temperature, still considerable more phosphoric acid was made available than in the check cultures. The mixtures incubated at room temperature to which sulfurous acid was added at the beginning showed up very favoeably in comparison with culture number one which received no sulfurous acid and was incubated at 30°C. It should be remarked here that a similar strong culture for inoculation was used as mentioned in a previous series. The addition of sulfurous acid by stirring into the mixtures at the times of aeration proved not to be so effective in each case, showing that possible oxidition of sulfurous acid has but little, if any, effect. Since these studies were mainly concerned with reducing the first part of the incubation period before sufficient free acid or acid salts are produced to make P_2O_5 available, the experiments were not continued.

In a recent publication Lipman and Joffe (12) report that initial reaction is of no advantage when sulfuric acid is used. The mixtures worked with by these investigators had however a relative high hydrogen-ion concentration at the start, the soil compost having a pH value of 5.4 before additions of sulfuric acid were made. Since it appears that most time required in changing the hydrogen-ion concentration from 7.3 to 5.8, when a alkaline soil is used, this may be the cause for finding no advanfage in lowering the pH values through additions of sulfuric acid.

SERIES VII. AERATION.

One of the conclusions drawn by Mc Lean (9) is that the question of aeration should receive foremost consideration. He states that the results obtained would make it appear that the micro-organisms which oxidize sulfur are largely aerobic, and hence require an abundant supply of oxygen. The experiments of Shedd (15) show that the stirring of the mixtures had considerable influence, and he concludes that thorough aeration is one of the conditions which promotes most rapid reaction.

Two series of experiments were conducted for the purpose of testing the influence of aeration. The first series was composed of 80 parts of rich alkaline garden soil, 100 parts of sulfur and 400 parts of rock phosphate. To the mixtures were added different agredients and the cultures placed in a dark cubboard at room temperature. The cultures were divided into two parts. One part was stirred twice a week by removing them from the glasses and mixing thoroughly, while the other part was stirred in the same way at the end of six weeks when a sample was taken for analyses.

Moisture contents were kept at the optimum by weighing at the end of every week. Although all tumblers were kept covered with glassplates, the amounts of water lost from the stirred cultures were far greater than from the cultures whish were not removed from the glasses. The results obtained after 6 and 12 weeks are reported in table 11.

Influence of aeration in the presence of different ingredients on the production of acidity and available P_2O_5

STIRRED TWICE A WEEK.

		After								
		0 weeks			6 weeks			12 weeks		
No	Additions	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5	Acidity	PH	% Sol. P_2O_5
1	None	Alk. .18	7.2	.00	1.7	5.8	.00	31.3	4.3	2.27
2	14 cc $(NH_4)_2SO_4$	Alk. .18	7.2	.00	4.9	5.2	.00	54.5	3.9	5.96
3	13 cc SO_2 in Water	.46	6.6	.00	32.7	5.1	.08	137.0	3.2	10.40
4	.01 % Na H CO_3	Alk. .04	7.1	.00	1.0	5.9	.00	20.3	5.4	1.51
5	6 cc Norm. CH_3COOH	Alk. .14	7.0	.00	2.5	5.8	.00	5.7	5.6	.00
6	.02% $Al_2(SO_4)_3$ and .02% Fe SO_4	Alk. .18	7.2	.00	1.5	5.9	.00	27.4	4.1	1.89

TABLE 11 CONTINUED.

		After								
		0 weeks			6 weeks			12 weeks		
No.	Additions	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5	Acid-ity	PH	% Sol. P_2O_5
7	None	Alk. .18	7.2	.00	.6	5.8	.00	8.2	5.5	.68
8	14 cc Norm. H_2SO_4	Alk. .18	7.2	.00	3.4	5.4	.00	44.4	3.9	4.08
9	13 cc SO_2 in water	.46	6.6	.00	8.7	5.2	.00	71.6	3.5	6.51
10	.01 % Na H CO_3	.04	7.1	.00	.8	5.9	.00	4.7	5.7	.00
11	6 cc Norm. CH_3COOH	Alk. .14	7.0	.00	.6	6.0	.00	1.9	5.9	.00
12	.02% $Al_2(SO_4)_3$ and .02% Fe SO_4	Alc. .18	7.2	.00	.7	5.8	.00	9.3	5.4	.76

STIRRED ONCE EVERY SIX WEEKS.

In this series of cultures the same was found as in earlier series regarding the influence of sulfuric acid. Sulfuric acid caused considerable stimulation of bacterial activities in the stirred and unstirred mixtures, resulting in relatively higher acidity and greater availability of P_2O_5 as compared with the check.

Sulfurous acid exerted again marked influence, while sodium bi-carbonate added in solution to this rich soil compost did not stimulate, but seemed to retard the activities of the sulfur oxidizing organisms. This would indicate that the conclusion drawn for the soil poor in organic material and concequently poor in carbon di-oxid production was right. In this soil was apperently sufficient CO_2 present for the work of the organisms and an addition of a CO_2 source had no influence.

Acetic acid proved to be in this soil detremental, and a combination of 0.02 per cent alluminum sulfate and 0.02 per cent of ferous sulfate had no influence. These two salts had no influence upon the activities of the organisms in any of the calcereous soils used.

All stirred mixtures were considerably in advange as compared with the unstirred mixtures. The conclusion drawn by McLean and Shedd that an abundance of oxygen favored sulfur oxidation seemed right. It was thought, therefore, that a still greater abundance of oxygen would help to accelerate the reaction still more rapidly. An apparatus was made in such a way as to have a small contineous stream of moist air running through the cultures. A diagram of the apparatus used is shown in figure III.

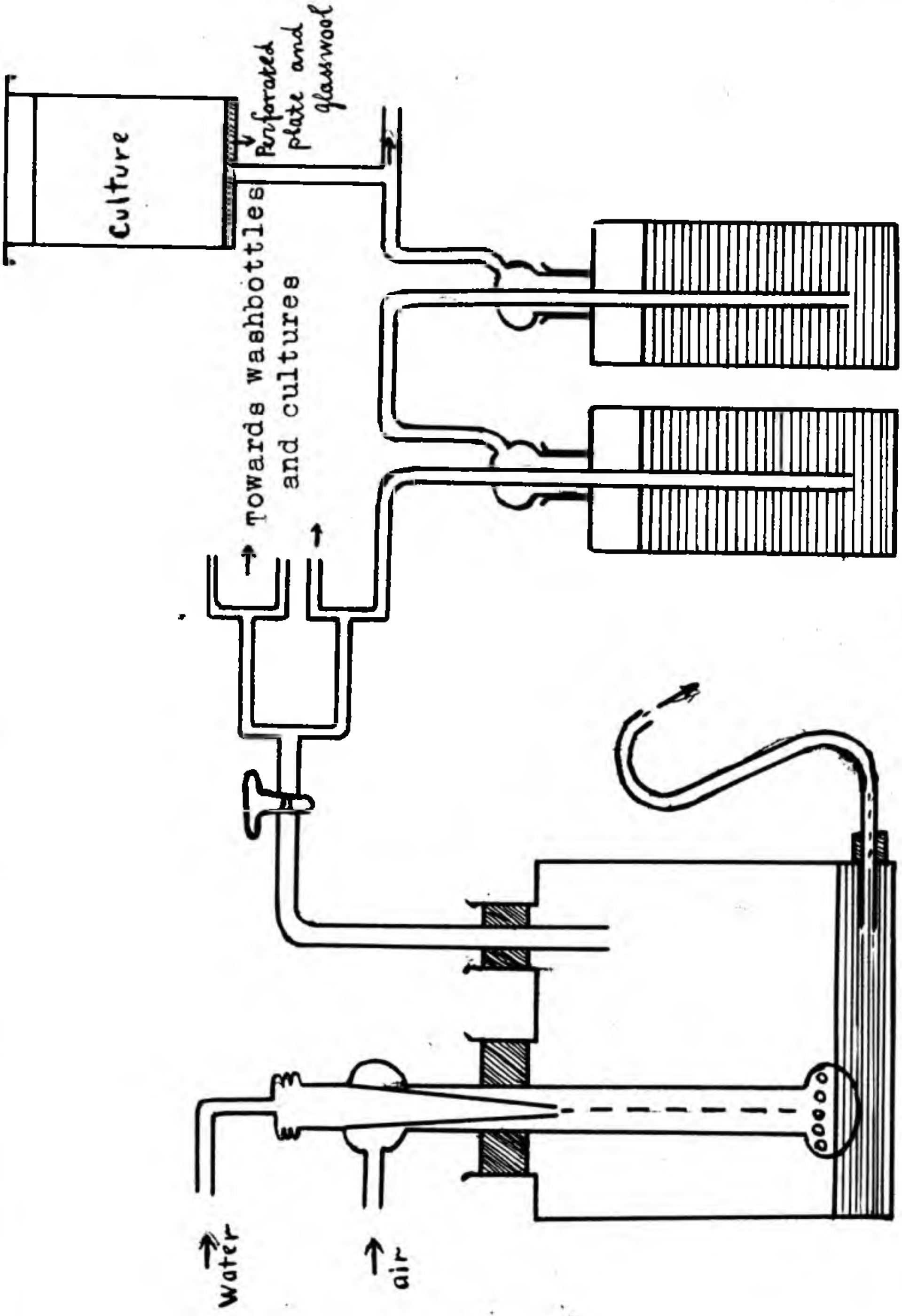
Culture
Perforated plate and glasswool
Towards washbottles and cultures
water
air

Fig. III.

As a soil compost mixture 10 parts of a neutal calcereous soil, 100 parts of sulfur, and 400 parts of rock phosphate was used. To replace the bulk of the soil a number of cultures received 0.2 ammonium sulfate. The water holding capacity of this mixture was 22 per cent.

To some of the cultures 10 cc. sulfurous acid in addition was given, while others received the same amount of sulfurous acid at the time of stirring for aeation. Some of the mixtures through which air was running received a similar amount of sulfurous acid at the start and the others by means of moist air which went through a washbottle containing the calculated amounts of weak sulfurous acid per 100 gram mixture.

The mixtures through which air was running were taken out of the containers and thotoughly mixed at the end of every two weeks. The cultures in the tumblers were stirred once at the end of each week. Preliminary experiments were made to determine the amounts and rate of air flowing through the mixtures. It was concluded best to use approximatily 1 liter of air per hour per 500 gram mixture. All mixtures were kept at room temperature. Acidity and H-ion concentration determinations were made every two weeks, and the soluble phosphoric acid at the end of 14 weeks. The results obtained are recorded in table 12.

TABLE 12

Influence of aeration on the change of hydrogen-ion concentration, production of acidity and soluble P_2O_5 .

		After									
		0 weeks		2 weeks		4 weeks		6 weeks		8 weeks	
No.	Additions	Acidity	PH	Acidity	PH	Acidity	PH	Acidity	PH	Acidity	PH
1	None. stream of moist air	.4	6.8	0	6.9	Alc. .2	7.0	0	6.9	.8	6.8
2	.2% $(NH_4)_2SO_4$; stream of moist air	.4	6.8	14.1	4.9	13.7	4.9	11.2	5.1	9.6	5.7
3	.2% $(NH_4)_2SO_4$; 10cc SO_2 in water added at begin; stream of moist air.	1.6	6.4	15.6	4.7	16.0	4.7	11.0	5.1	9.1	5.7
4	.2% $(NH_4)_2SO_4$; 10cc SO_2 in water added to container through which air was running.	1.6	6.4	80.4	4.5	72.2	4.7	71.0	3.6	65.5	4.1
5	None	.4	6.8	.8	6.7	1.0	6.2	1.0	5.7	1.6	5.7
6	.2% $(NH_4)_2SO_4$	.4	6.8	54.9	4.6	49.8	4.7	57.7	3.8	65.4	3.8
7	.2% $(NH_4)_2SO_4$; 10 cc SO_2 in water added at begin.	1.6	6.4	55.7	4.6	49.8	4.9	57.4	3.7	95.2	3.5
8	.2% $(NH_4)_2$ SO_4; 10 cc SO_2 in water added by stirring	1.6	6.4	54.7	4.7	48.8	4.8	59.8	3.8	97.5	3.5

TABLE 12 CONTINUED

		After						
		10 weeks		12 weeks		14 weeks		
No.	Additions	Acidity	PH	Acidity	PH	Acidity	PH	% Sol. P_2O_5
1	None; stream of moist air	.4	6.8	1.1	6.6	4.7	6.3	.00
2	.2% $(NH_4)_2$ SO_4;stream of moist air	10.8	5.6	16.5	5.3	9.5	5.5	.08
3	.2% $(NH_4)_2$ SO_4; 10cc SO_2 in water added at begin; stream of moist air.	7.6	5.7	13.3	5.5	5.7	5.9	.00
4	.2% $(NH_4)_2$ SO_4 ;10cc SO_2 in water added to container through which air was running	58.1	3.7	62.1	4.2	37.0	5.0	trace
5	None	3.2	5.6	14.6	4.9	16.1	4.8	.00
6	.2% $(NH_4)_2$ SO_4	131.7	3.2	154.0	3.3	170.0	3.0	9.66
7	.2% $(NH_4)_2$ SO_4 ;10 cc SO_2 in water added at begin.	141.7	3.1	165.9	3.3	180.5	3.0	10.70
8	.2% $(NH_4)_2$ SO_4 ; 10 cc SO_2 in water added by stirring.	152.4	3.1	174.2	3.3	190.0	3.1	9.86

While the difference between stirred and unstirred mixtures was considerable, the difference between aerated and stirred composts was still greater, Here,however,the difference was in the opposite direction. All aerated mixtures produced not only less relative acidity,but the available phosphoric acid was nill in the aerated cultures (numbers 1 to 4) after 14 weeks. The aerated mixtures produced after two weeks some activity but this activity decreased as time went on. In the case of number 4 a part of the measured acidity in all probability was free sulfurous acid which went through,as indicated both by titration and H-ion concentratinn.Every four weeks moisture determinations were made on the mixtures. These determinations showed that the optimum moisture content was very constant.

The stirred mixtures increased regularly in acidity, while the mixtures to which ammonium sulfate was added, in spite of the neutral calcereous soil,produced from the very beginning a much greater acidity than the check cultures without ammonium sulfate. The soluble P_2O_5 in these mixtures at the end of 14 weeks was approximately 10 per cent,all being close together as well in acidity as in available phosphoric acid.

There seems no doubt but that too great a quantity of air does not favor sulfur oxidation by the organisms as was supposed.

There seems to be a limit in the quantity of oxygen which acts favorably on the organisms,but where the limit lies could not be determined. The quantities of air which went through - namely 24 liters per day per 500 gram mixture - were apperantly too great,although 200 liter per hour per ton mixture does not seem such an extreme abundance.

OBSERVATIONS.

It was noticed throughout a number of series that the relative acidity regularly increased,but the intensity of theacid produced as measured by the H-ion concentration, varied. The cocentrations becoming higher,(lower pH values) till a cerain point (3.1 to 2.9) was reached,than getting slightly weaker and than back to the same point as before (3.1to 2.9) to remain there. This point seemed to indicate when the greatest production of available phosphoric acid began. The hydrogen-ion concentration however,does not necessarily have to be at a certain point,pH 3.2 for instance,to make phosphoric acid available.

By the measurements of relative acidity the soluble phosphates are included and does therefore not express the exact amounts of sulfates and free acid formed. The constant action of the free acid on the tri-calcium phosphate may account for the irrigularities noticed in the determinations of the hydrogen-ion concentrations. The point at which the greatest amounts of P_2O_5 are made available corresponds with the hydrogen-ion concentration of acid phosphate, which is in general from 3.0 to 3.2 as expressed in pH values.

Throughout this work it was frequently noticed that the mixtures had a tendency to form small aggregates composed of soil, rock phosphate and sulfur. Upon examination of these aggregates under the microscope they appeared to have a somewhat peculiar structure of indifinite form. A great number of these clodded particles were picked out and their relative acidity, hydrogen-ion concentration and available P_2O_5 determined. A part of the mixture was taken as ordinarily was done by determinations and was cautiously sifted. The differences found are tabulated in table 13.

Table 13.
Difference in size of aggregates and their relative acidity, pH values, and available P_2O_5 as compared with the mixed compost.

	acdity	pH	available P_2O_5 (per cent)
Coarse aggregates (± 4-5mm.diameter)	197.0	3.0	16.48
Medium size aggregates (-2-3 mm.diameter)	190.2	3.1	15.92
Small aggreagtes (± 1 mm.diameter)	185.6	3.1	12.38
Fine (sifted)	121.8	3.3	8.86
Mixture	171.0	3.1	11.42

On account of this peculiarity these aggregates or crumbs were later on broken up when the composts were mixed and when samples were taken for analyses.

Often it was found that the incubated mixtures produced hydrogen sulfide during the first weeks of the incubation period. Sometimes if was only noticeable by the smell and at other times even by a black colour produced. Small additions of sulfuric acid were able to help the sulfur oxidizing organisms,and depressed the hydrogen sulfide producing organisms.

CONCLUSIONS..

1. Sulfur oxidation takes place in a calcereous slightly alkaline soil and does not hinder the solubility of phosphorus when this soil is used in composts.

2. Composts mixtures incubated at 30°C increased more rapidly in relative acidity and available P_2O_5 than composts kept at room temperature.

   When large quantities of a calcereous soil is used the influence of temperature is usually greater than when small ampunts of such a soil are employed.

3. The influence of light is slightly detremental to sulfur oxidizing organisms.

4. a. Small quantities of sulfuric acid are stimulating bacterial activities particularly during the first weeks of the incubation period, if calcereous, slightly alkaline soil is used.

   b. Sodium bi-carbonate stimulated considerable bacterial activities when a calcereous soil poor in organic material was used; if a calcereous soil rich in organic material was used it failed to produce stimulation to the same extent, presumebly due to greater CO_2 production in this soil.

   Sodium bi-sulfate in solution and acetic acid had no stimulating effect, but were rather detremental.

c. A mixture of 0.02 per cent ferrous sulfate and 0.02 per cent aluminum sulfate failed to exert any influence.

5. Partial sterilisation of the soil and the mixtures by additions of sodium chloride and hydrochloric acid proved be of no value. NaCl retarded the activities of the sulfur oxidizing organisms, and the same was true to a less extent with HCl.

6. The amounts of soil can be reduced with success from 16 or 17 per cent to 1.6 or 1.7 per cent of the mixtures. It is possible to replace the bulk of a calcereous soil, poor in nitrgenous material with ammonium sulfate.

7. The quantities of sulfur in the mixtures, reduced to approximately the amounts required in ordinary factory methods of making acid phosphate, gave good results.

8. Addition of small amounts of sulfurous acid to change the initial reaction of these mixtures in which a slightly alkaline calcereous soil was used, proved to have a decided influence upon the rapidity of accumulation of acidity and available phosphoric acid.

9. Aeration of the mixtures had considerable influence, but when the mixtures received an abundance of air, sulfur oxidation nearly ceased and no phosphoric acid was made available

10. The hydrogen-ion concentration in the mixtures changed till pH values of from 3.1 to 2.9 were reached, indicating the point at which most P_2O_5 became available. Relative acidity as measured by the titration method ,accumulated after this point was reached.
11. This mixtures have a tendency to form aggregates which have a different relative acidity and different quantities of available P_2O_5 according to the size of the aggregates.

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PART TWO.

THE OXIDATION OF IRON SULFIDES BY SULFUR OXIDIZING ORGANISMS AND THE USE OF PYRITES IN COMPOST MIXTURES FOR MAKING MINERAL PHOSPHATES AVAILABLE.

Intrduction.

It is very well known among sulfuric acid manufacturers and others that iron sulfides (pyrites) tend to oxidize slowly into sulfates. These changes seem to be more rapid when these pyrites are moist or laying on the ground. They first oxidize,forming ferrous sulfate,and since this compound is not stable in presence of water or moist air,this is converted into basic iron sulfates and then into iron hydroxids which are but slightly soluble.

Löhnis (3) discribing the influence of micro organisms on the sulfur cycle says (p.705) that thry do the principal work.The sulfides are tranformed into Hydrogen sulfide;the same may happen with the thiosulfates and more seldom with the sulfates.The H_2S and the thiosulfate are oxidized into sulfates. At another place however,(708) he lays emphasis on the fact that the oxygen of the air has a strong oxidizing action. Allen and Johnston (1) found that when pyrites are ground for analyses they suffer partial oxidation to sulfur dioxide and ferrous sulfate.Whether or not this action is of a purely chemical nature Kappen and Quensel (5) have tried to acertain. They weighed into a porcelan dish, in the bottom of which was a filter paper, 100 grams of sandy soil, moistened with 15 cc. of water, and drained off the surplus with asuction pump. Hydrogen sulfide was passed through the soil till the mass had ablack colour. The soil was then placed in the open air and in 30 minutes the colour had changed and the iron sulfide changed into iron sulfates. After standing for 5 hours the soil was treated with 10 per cent HCl and the amount of sulfuric acid determined in the extract. For 100 grams of untreated soil they fouhd as an average 2.11 mgr. of sulfur as sulfates at the beginning and 2.34 mgr. after 5 hours. With the treated soil they found 4.15 mgr. at the beginning and 5.01 mgr. after 5 hours. They draw the conclusion "dass überal da, wo sich im boden durch Faulnis von Eiweisstoffen oder durch Reduktion von Sulfaten Schwefelwasserstoff bildet, or sofort durch dass wohl in allen Böden in dazu genügenden Mengen vorhandene Eisenoxydhydrat unter Schwefelabscheidung und Reduktion des Eisenoxydes gebunden wird, und dass das Schwefeleisen, die Zutrittsmöglichkeit von Sauerstoff naturlich vorausgesetzt, sich ohne Einwirkung von Mikroorganismen weiterzersetzt. Die höchste Oxydationsstufe des Schwefels, die wieder ohne weiteres in den Kreislauf eintreten könnte, entsteht hierbei nicht, oder nur in Spuren; es bildet sich vielmehr hierbei

fast ausschliesslich elementarer Schwefel." Their further studies led them to conclude that this elementary sulfur is changed to sulfates by various influences. Van Bemmelen (7) pointed out that pyrites occurs locally in diatomes and also in plant cells.He explains the formation of pyrites as a sulfate reduction.The iron in the soil is changed into ironsulfates in the presence of sulfates.By the oxidation of the iron sulfite sulfuric acid is formed.This sulfuric acid does not attack,or but very slightly attacks,the silicates in the soil,(Van Bemmelen p.81,85,97,98) but does act upon the irom oxid present. This investigater concludes (97) "That the ironoxid in the soil protects,to a certain extent, the humate and silicate bases against the action of the sulfuris acid." After the oxidarion of the sulfur to sulfuric acid an ironsulfate is formed from this acid and the iron.

PURPOSE OF INVESTIGATION.

From the work of Kappen and Quensel, Allen and Johnston and others it could be concluded or is at least doubtful that the action of bacteria on the transformation of iron sulfide into iron sulfate is slight or negligeable.

Two main points were open for investigation:

A. Do sulfur oxidizing organisms transform iron sulfide into iron sulfate,

B. If so, what is the influence on the availability of rock phosphate composted with a mixture of pyrites, sulfur and soil.

Experimental results.

Series I.

A slightly acid air dry soil was secured and mixed thoroughly with not very finely ground iron pyrites, This mixture was divided into two parts of which one part was inoculated with a soil compost extract known to contain sulfur oxidizing organisms. An equal amount of a slightly acid air dry soil was mixed with flowers of sulfur, divided, and one part treated in a similar way as is described above. The mixture contained 50 parts of soil and 100 parts of sulfur, while the amounts of pyrites used were calculated to contain the same quantities of sulfur. The iron pyrites consisted of 45.6 per cent of sulfur and 47.8 per cent of iron. The water holding capacity of the mixtures was determined according to the Hilgard method and the moisture content kept at the supposed optimum of 60 per cent of the water holding capacity by adding twice a week the amounts of water lost. The mixtures were placed in tumblers covered with glass-plates, stirred once every two weeks and kept at 28°C during the incubation period of 14 weeks. Determinations of hydrogen-ion concentration, relative acidity and water soluble sulfates were made at intervals of two weeks. The relative acidity and H-ion concentrations were determined in the manner described in part I. The water soluble sulfates were determined in the following way:

The composts were thoroughly mixed and 4 grams transferred to a 500 cc. flask with about 200 cc. distilled water and 5 cc. HCl (22°B). The flasks were vigorouely shaken and left standing for 12 hours; filtered into lasks of a 250 cc. capacity, an aliqot drawn off and precipitated with Barymm-chloride. The precipitate was filtered off, washed and calcinated, dried and weighed as Baryum sulfate.

The data secured are given in table 1.

TABLE I

Comparative effect of inoculation on the production of water soluble sulfates in a mixture of soil-sulfur and a soil-iron sulfide mixture.

	Pyrite							Sulfur						
	Not Inoculated			Inoculated				Not Inoculated			Inoculated			
Weeks	PH	Acid-ity	% Sol. Sulfate	PH	Acid-ity	% Sol. Sulfate	Over Check	PH	Acid-ity	% Sol. Sulfate	PH	Acid-ity	% Sol. Sulfate	Over Check
0	6.1	8.9	.63	6.1	8.9	.63		4.6	23.4	.13	4.6	23.4	.13	
2	6.0	9.2	.88	5.7	14.2	1.96	1.08	4.5	24.8	.12	3.9	36.1	.88	.66
4	6.0	9.4	.97	4.4	16.2	2.47	1.50	4.7	26.2	.16	2.6	172.1	2.32	1.16
8	5.9	9.7	1.12	3.6	20.5	3.27	2.15	4.4	25.8	.15	2.1	279.5	2.82	2.67
12	5.8	10.9	1.26	3.9	28.3	4.12	2.86	4.4	26.0	.19	2.0	322.2	3.22	3.03
14	5.7	12.2	1.38	4.2	47.5	4.65	3.27	4.4	27.2	.21	2.1	305.9	3.66	3.45

It is evedent that under these conditions oxidation of iron pyrites took place in the uninoculated mixtures, as is indicated in the change of hydrogen-ion concentration, the increase in relative acidity and the per cent of water soluble sulfates formed after 14 weeks. The increase was gradual, as was the case in the inoculated mixtures. However, the change in pH values in the inoculated soil-iron pyrites mixture was considerably greater, and the relative acidity increased more rapidly. The water soluble sulfates formed had increased at the end of 14 weeks tomore than 3 times the sulfates formed in the uninoculated mixture. The total water soluble sulfates of the uninoculated mixture after 14 weeks was but 1.38 per cent of the sulfur present, while the total water soluble sulfates of the inoculated mixture after the same time of incubation had increased to 4.65 per cent of the sulfur present. The mixtures were less frequently aerated in order to see whether or not the inoculated mixture would produce more sulfates under the circumstances than the uninoculated pyrite-soil mixture.

The pH values in this inoculated mixture went down (H-ion concentration increased) to 3.6 after 8 weeks and from then on went up again, undoubtedly because the action of the acid formed upon the pyrites, constitutes a buffer action.

In The uninoculated soil-sulfur mixture the pH value went down gradually also, accompanied by a slight increase in relative acidity and water soluble sulfates. It has to be stated here that it was difficult to keep the uninoculated mixtures free from contemination. It is possible therefore that in both cases the uninoculated mixtures contained after some time some sulfur oxidizing organisms. The inoculated sulfur-soil mixture increased rapidly in acidity as shown by the higher hydrogen-ion concentration and titrated acidity. The sulfur present was oxidized at nearly the same rate as in the case of the inoculated iron pyrites soil mixture, the former being but 0.18 per cent more at the end of 14 weeks. If the mixtures had been more frequently aerated this would have been greater as indicated by other experiments. All mixtures remained closely packed in the tumblers and were not stirred except for taking samples.

As could be expected the hydrogen-ion concentration was considerably higher on account of there being no material present except the soil constituents for the sulfuric acid to react with. No attempt was made, however, to determine the possible increase in soluble potassium, phosphorus, etc. present in the soil.

From the results obtained the conclusion was drawn that the proper sulfur oxidizing organisms are active in the transformations of pyrites when these are mixed with soil and kept at a supposed optimum moisture content.

SERIES II. PYRITES COMPOSTED WITH A MIXTURE OF SOIL-SULFER ROCK PHOSPHATE.

As has been pointed out it was interesting to find out what the influence of iron pyrites would be on the availability of rock phosphate when composted with a soil and sulfur mixture. A series of experiments was conducted in which 100 parts of soil were mixed with 400 parts of rock phosphate and different amounts of sulfur and pyrites. For this purpose aslightly alkaline calcereous soil was used and the sulfur replaced by pyrites so as to have approximately the same amounts of sulfur in all experiments. The exact quantities of pyrites used together with the data secured in 12 weeks are given in table 2. The relative acidity and hydrogen-ion concentration was determined at intervals and the available P_2O_5 determined after 9 and after 12 weeks. One series of cultures was kept at room temperature and another series with the same additions incubated in darkness at 30°C.

Influence of iron pyrite composted with a mixture of soil-sulfur - rock-phosphate on the availability of phosphoric acid.

No.	Additions	After													
		0 weeks		3 weeks		5 weeks		7 weeks		9 weeks			12 weeks		
		PH	Acidity	PH	Acidity	PH	Acidity	PH	Acidity	PH	Acidity	% Sol. P_2O_5	PH	Acidity	% Sol. P_2O_5
1	90 sulfur 60 pyrite	6.9	.0	5.6	1.7	4.2	52.4	3.2	169.5	3.1	178.0	9.39	3.0	198.4	10.62
2	80 sulfur 80 pyrite	6.9	.0	5.2	4.9	4.2	51.1	3.3	162.4	3.1	174.0	8.93	3.1	189.8	9.52
3	70 sulfur 100 pyrite	6.9	.0	5.4	2.4	4.1	55.1	3.4	136.6	3.3	152.0	8.28	3.1	175.3	9.94
4	60 sulfur 120 pyrite	6.9	.0	5.3	3.9	4.1	58.3	3.3	159.0	3.0	171.0	9.20	3.0	189.4	10.62
5	120 sulfur	6.9	.0	5.8	3.8	4.2	52.1	3.4	148.1	3.2	161.2	8.88	3.0	180.1	9.98

at 30 °C

TABLE 2 CONTINUED

			After													
			0 weeks		3 weeks		5 weeks		7 weeks		9 weeks			12 weeks		
No.	Additions		PH	Acid-ity	PH	Acid-ity	PH	Acid-ity	PH	Acid-ity	PH	Acid-ity	% Sol. P_2O_5	PH	Acid-ity	% Sol. P_2O_5
6	90 sulfur 60 pyrite	At Room Temperature.	6.9	.0	5.9	.8	5.8	2.8	5.6	4.3	5.6	7.6	.00	4.6	48.7	.76
7	80 sulfur 80 pyrite		6.9	.0	6.0	.6	5.9	2.7	5.5	8.7	5.4	16.5	.00	4.0	53.2	1.96
8	70 sulfur 100 pyrite		6.9	.0	6.0	.8	5.8	3.1	5.6	7.6	4.9	14.4	.00	3.7	48.6	2.02
9	60 sulfur 120 pyrite		6.9	.0	6.0	.8	5.8	2.4	5.6	5.3	5.2	10.1	.00	3.8	21.5	1.88
10	120 sulfur		6.9	.0	5.9	.8	5.8	2.8	5.5	7.8	5.0	14.2	.00	3.7	49.4	1.98

Table 2 shows that the pH values for the cultures incubated at 30°C had after 5 weeks reached the point at which the phosphoric acid becomes available. The relative acidity increased rapidly until the end of 7 weeks but from then on increased less rapid. This could be expected on account of the neutralisation of the acid by the tri-calcium phosphate. present. At the end of 9 weeks and at the end of 12 weeks there was no appreciable difference in total soluble P_2O_5 and acidity inany of these cultures, and did noy appreciably differ from the cultures without pyrites used as checks. The same was true in the cultures kept at room temperature. The influence of temperature was very pronounced in these cultures. In the cultures incubated at room temperature but little P_2O_5 had been made soluble after a period of 12 weeks. However, the accumulation of acidity in the mixtures with pyrites was as gradual as in the mixtures without pyrites, being approximately the same in most of the cultures. It seemed therefore that the iron pyrites did not interfere with the formation of sulfates, nor with the availability of phosphoric acid.

Series III. Substitution of Ammonium sulfate for Soil.

A series of experiments similar to those as reported in part I, table 8, was conducted with a mixture of 10 parts of

Soil,400 parts of rock phosphate, 50 parts of sulfur and 100 parts of iron sulphide,in which the great bulk of soil was replaced by ammonium sulfate. The mixtsrs were incubated at 30°C and at room temperature. Two tumblers in duplicate received 0.2 per cent of ammonium sulfate and to two of them was added also at the beginning of the incubation period 10 cc. sulfuric acid per 100 grams of mixture. The pyrites had a lower pH value than the soil and rock phosphate,which resulted in making the mixtures slightly acid. Relative acidity and hydrogen-ion concentration was determined at intervals of two weeks and available phosphoric acid at the end of 10 weeks. The results are reported in table 3.

TABLE 3

Replacement of soil by ammonium sulfate in composts in which quantities of Sulfurare replaced by iron sulphide.

		After												
		0 weeks		2 weeks		4 weeks		6 weeks		10 weeks			12 weeks	
No.	Additions	Acid-ity	PH	Acid-ity	PH	Acid-ity	PH	Acid-ity	PH	Acid-ity	PH	Sol. P_2O_5 %	Acid-ity	PH
1	None, at $30^{\circ}C$	.2	6.9	2.8	6.4	41.2	4.2	74.5	3.8	92.1	3.8	5.12	101.2	4.0
2	0.2% $(NH_4)_2SO_4$ at $30^{\circ}C$.	.6	6.7	43.5	4.1	76.7	3.8	127.3	3.6	132.2	3.3	9.54	153.9	3.9
3	None, Roomtemperature	.2	6.9	1.9	6.6	20.6	5.2	37.0	4.1	87.0	3.8	4.86	99.7	4.1
4	0.2% $(NH_4)_2SO_4$ Room Temperature	.6	6.7	8.9	5.5	44.7	4.1	45.6	4.0	115.5	3.4	6.26	159.6	3.7
5	0.2% $(NH_4)_2SO_4$, 10 cc H_2SO_4 per 100 gram mixture. Room Temperature	1.8	6.6	2.8	6.4	43.7	4.5	44.6	4.0	116.5	3.4	6.58	140.6	3.8

The influence of ammonium sulfate was noticeable from the beginning in the cultures kept at room temperature as well as in the cultures incubated at 30°C. The composts incubated at 30°C which received 0.2 per cent of ammonium sulfate had accumulated an acidity at the end of 10 weeks aquivalent to 132.2 cc. of 0.5 norm.sodium hydroxid as against an acidity aquivalent of 92.1 cc.0.5 norm.NaOH accumulated by the cultures with no ammonium sulfate.

The available phosphoric acid for these mixtures at the end of the same period was 9.54 per cent and 5.2 per cent respectivily.

Here again the strenght of the acid formed,as indicated by the lower pH values after 10 weeks of incubation,was greater than after 12 weeks,athough the total acidity had increased considerably in the period between 10 and 12 weeks.It was at first thought that this was a mistake,but the figures given represent an average of 6 determinations. The measurement of hydrogen-ion concentration is a measuring of free acid at the time of the determination,and this change continously. Besides,the buffer action in the mixture may cause different readings at different times. Hydrogen-ion concentration measurements may indicate at which point of acidity accumulation,phosphoric acid becomes available and are,as such,afford a means to follow the progress of bacterial activities,but they have,naturally,no value as to the quantities of acid and acid salts produced.

SERIES IV. AERATION.

The aeration experiments reported in part I were repeated with a compost consisting of the same amounts of soil, rock phosphate,sulfur,pyrites and ammonium sulfate as were used in series III. In addition, some of the mixtures which were contineously aerated received 10 cc. of sulfurous acid per 100 grams of the mixtures at the beginning of the experiment, and other received similar quantities of sulfurous acid in the stream of moist air used for aeration. The apparatus shown in fig,I of part I was used the provide the mixtures with moist air, The mixtures which were placed in tumblers and to which 10 cc. sulfurous acid per 100 grams of mixture were added,received these quantities at the beginning or by stirring into the mixtures during the first 8 weeks. All composts were placed at room temperature and kept in darkness

The results obtained are reported in table 4.

TABLE 4

Influence of continuous aeration on the accumulation of acidity and available phosphoric acid.

No.	Additions	After 0 weeks Acidity	 PH	 2 weeks Acidity	 PH	 4 weeks Acidity	 PH	 6 weeks Acidity	 PH	 8 weeks Acidity	 PH	 10 weeks Acidity	 PH	 12 weeks Acidity	 PH	 % Sol. P_2O_5
1	None, stream of moist air	.6	6.7	4.6	5.9	12.4	5.6	16.8	5.1	22.4	5.0	36.2	4.8	59.4	4.2	.62
2	0.2% $(NH_4)_2SO_4$; stream of moist air.	.6	6.7	47.1	4.4	44.1	4.7	82.1	3.6	130.1	3.5	141.5	3.2	163.6	3.4	10.45
3	0.2% $(NH_4)_2SO_4$; 10 cc Sulfurous acid added at begin. stream of moist air.	.2	6.9	2.5	5.9	39.7	4.8	23.0	5.0	120.0	3.6	130.1	3.2	145.5	3.5	8.26
4	0.2% $(NH_4)_2SO_4$; 10 cc Sulfurous acid added to container through which air was running.	.2	6.9	70.1	4.3	66.7	5.0	65.2	3.7	59.8	3.9	57.4	3.8	62.5	4.0	.78
5	None	.6	6.7	.6	6.6	1.0	6.4	2.8	5.5	16.1	5.0	34.0	3.9	51.1	4.0	.32
6	0.2% $(NH_4)_2SO_4$	.6	6.7	45.8	4.5	44.3	4.7	52.4	3.9	94.6	3.6	120.3	3.3	153.3	3.4	9.32
7	0.2% $(NH_4)_2SO_4$; 10 cc Sulfurous acid added at begin.	.2	6.9	4.0	5.7	42.8	4.8	57.1	3.9	49.7	3.8	53.2	3.9	130.4	3.5	7.81
8	0.2% $(NH_4)_2SO_4$; 10 cc Sulfurous acid added by stirring.	.2	6.9	3.4	6.0	36.1	4.9	47.9	3.9	44.4	3.9	47.9	4.0	123.9	3.8	5.76

It is evedent from table 4 that aeration of the mixtures by means of a stream of air had very little or no beneficial influence. Contrary to the experiments reported in table 12, part I, these aerated mixtures accumulated considerable acidity provided ammonium sulfate was added, These particular mixtures had made available an average of 10.45 per cent phosphoric acid at the end of 10 weeks. This could not be due to the abundance of air acting on the pyrites for the cultures without ammonium sulfate received exactly the same amounts of air. The effect is described to the pyrites acting as catalyser and thereby favoring the action of the sulfur oxidizing organisms. As evidence for this assumption it may be stated that qualitative analyses of the mixtures showed that great quantities of iron sulfates were present. This was undoubtedly due to the activities of the micro-organisms, for but comparatively small amounts of iron sulfates were present in mixtures numbers 1 and 6 which received no ammonium sulfates as source of nitrogen and which in consequence produced but little acidity.

The addition of sulfurous acid failed to stimulate these mixtures,neither in the composts contineously aerated nor in the mixtures kept in tumblers. Sulfurous acid stirred into the mixtures or added in the stream of moist air proved to be depressing rather than stimulating.

The action of sulfurous asid, threfore, seems to be in the first place of a sterilizing nature. Especially where small amounts of soil are used this action is more pronounced.

SERIES V. VEGETATION EXPERIMENTS.

Since iron is one of the absolutely essential elements to normal growth and development of all agricultural plants, but since the quantities of iron in the soil are usually so large and supposed to be available in sufficient amounts to perform the necessary functions, it is not often applied as a fertilizer.

Streneous efforts have been made by the manufacturers of certain products to introduce iron sulfate, which is a common byproduct of a number of manufacturing processes, as a fertilizer. Pyrite cindres have been used in many places with success, especially in the Aisne region in France. Their value has been attributed both to the iron sulfate and to small amounts of nitrogen which these cindres contain.

Vivien (9) mixed one per cent of roasted pyrites with manure and found that considerable amounts of nitrates were formed, whereas in manure treated with acid phosphate, iron sulfate, calcium sulfate, and lime, only traces of nitrates were found.

Vermorel and Dantony (8) employed iron pyrites at rates of 100 and 200 kilograms per hectare as a top-dressing for wheat and beans. In one series they employed pure sand with additions of 50 kilograms sodium nitrate per hectare and in another series 100 kilograms dried blood per hectare. The pyrites was given as a top dressing and mixed with the sand.

The pyrites increased the yields of wheat 40 per cent and for beans 50 per cent. These investigators conclude that the sulfur of the pyrites acted as a stimulant. They found however, that pyrites alone was more effective than sulfur alone on wheat and less effective on beans. Best results were obtained with a mixture of sulfur and pyrites.

Since these investigators had better results with a mixture of pyrites and sulfur the question can be raised whether this was due to the iron in a form more available to the plants. Van Alstine (6) concludes from his solution culture work that with a limited supply of iron in the form of ferri hydroxy-phosphate "a H-ion concentration of 4.5 is as low as can be expected to dissolve the amounts of iron necessary for proper growth of buckwheat, soy beans and wheat. With H-ion concentration (higher pH values) so little iron is dissolved that these plants are unable to get the amounts they need and begin to show chlorosis as soon as the supply in the seed is used up." The form in which iron is supplied is very important as is shown by Jones and Shive (4) in their nutrient solution work. In the nutrient solution employed, iron in the form of ferri sulfate was very slowly and difficulty available to wheat plants even when supplied in relatively large quantities, but ferrous sulfate appeared to be readily available to these plants. It might be that the good results of Vermorel and Dantony with pyrites were caused by the iron changing from the sulfide into the sulfate form and thereby becoming more readily available, for they used parafined pots with certain quantities of pure sand which was carefully freed from organic material. Through the washing of the sand they had naturally removed the iron present. That sufficient iron in the soil is not always available to plant growth is shown by the results obtained by Chanzit (2) with vines which showed chlorosis. He found that the prsence of excessive amounts of $CaCO_3$ in the soil caused chlorosis to the vines, which he could overcome by applying 250 -300 grams of ferrous sulfate to each vine during the winter.

An experiment was carried on in the greenhouse in earthenware pots with washed quartz sand. Shive's nutrient solution ($R5C_2$) was used as basis. The following materials were used; (the numbers refer to the pot cultures):

1. None
2. Pyrites
4. Nutrient solution (R5C2)
5. Nutrient solution and pyrites

6. Nutrient solution, but phosphorus replaced by ground rock phosphate
7. Nutrient solution, but phosphorus replaced by ground rock phosphate, and pyrites
9. Nutrient soltion, but phosphorus replaced by ground rock phosphate, inoculated sulfur, and pyrites.

The pyrites were added at a rate of 200 pounds per acre. The amount of rock phosphate employed was 2 tons per acre, calculated to be approximately the same as in the nutrient solution. Sulfur was added at a rate of 100 pounds per acre.

Soy beans were grown for 6 weeks and the yields obtained are recorded in table 5, together with other data obtained.

Table 5

Yields of soy bean tops grown in sand cultures with Shive's nutrient solution as basis, and with additions of pyrites.

No	Materials added	Dry weight in gr.	Avarage height incm.	Number of pods	pH begin	pH end
1	None	0.850	8	Bloom	5.8	6.7
2	Pyrites	0.990	7.5	Bloom	5.4	6.5
4	R5C2	3.342	14	3	5.4	6.5
5	R5c2, pyrites	3.610	15	9	5.2	6.6
7	R5C2, rock phosphate pyites	2.022	10.5	2	4.9	6.4
9	R5C2, R.P., Sulfur, pyrites	0.704	dead	-	5.0	3.3

Although the yields of tops were but slightly higher for the cultures receiving pyrites in addition to the nutrient solution,they were markedly earlier in maturing. After 4 weeks all plants to which pyrites were added were blooming,while but one plant of the other cultures started to bloom at that time. This earlier maturing is also indicated by the number of pods produced after 6 weeks. The plants receiving inoculated sulfur and pyrites were dead after 18 days. The acidity produced being apparently tooh high for these plants.

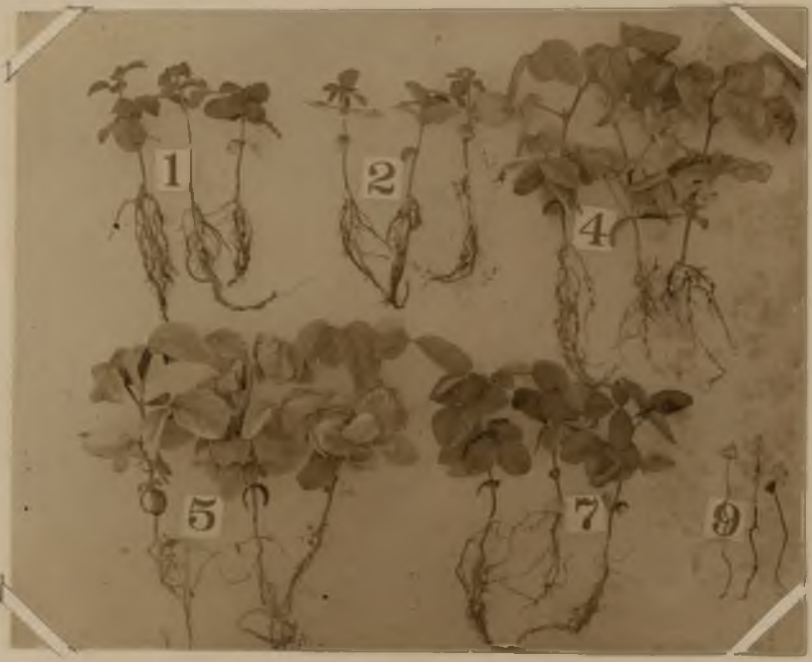

Fig.1. Soy bean plants grown in sand cultures with Shive's nutrient solution as a basis,and additions of pyrites.

Although no endeavor could be made to draw conclusions from these experiments, since they were of too limited a scope, they seem to point to the possibility that at least a part of the increase in yields obtained by Vermorel and Dantony is caused by a change of the iron to more forms, since no iron was applied at the beginning of the experiments, conducted by these investigators.

CONCLUSIONS.

1. From these studies it seems evident that iron sulfide (pyrites) can be attacked by microorganisms and changed into the sulfate form. No attempt however, was made to study the intermediate steps in the changes occurring. If small quantities of sulfur are added these changes are much more rapid.

2. Pyrites composted together with sulfur and rock phosphate do not interfere with the gradual increase in acidity formation nor with the increase in availability of phosphoric acid.

3. The replacement of soil with ammonium sulfate in composts in which quantities of sulfur are replaced by iron sulfide produced a marked increase in available phosphoric acid. The effect when sufficient nitrogen is present for the needs of the micro-organisms, is asribed partly to the pyrites acting

as a aatalyser and as such favoring the action of sulfur oxidizing organisms,and partly to the changes from the sulfide into the sulfate form.

4. Aeration of sulfur-pyrites-rock phosphate compost mixtures by means of a contineous stream of air has little or no beneficial effect upon the production of acidity and consequent availability of soluble P_2O_5,unless ammonium sulfate is added.

5. The action of sulfurous acid in such mixtures seem to be mainly of a sterilizing nature.

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PART THREE.

THE RELATION BETWEEN SULFUR OXIDATION TO THE AVAILABILITY OF THE POTASSIUM IN GREENSAND.

Athough a study of the literature shows that the application of greensand for agriculture purposes has proven beneficial and although good results by the use of greensand were recognized long ago in England and France and later in America, especially in New Jersey, farmers did not turn to these great resources even during war times when potassium was to be had only with difficulty. This is undoubtedly due to the fact that all greensands found in America have but a low percentage of potassium, and to the ease with which the farmer in ordinary times can buy readily available potash. The potassium of the greensand is but slowly available for plant food, and the value of the greensand as a fertilizer has been, and still often is, ascribed to the calcium carbonate and phosphorus present. Since a large number of the New Jersey greensands contain from 1 to 3 per cent of phosphoric acid and from 4 to 6 per cent potassium, it is probably that the beneficial effects can be attributed to both these constituents, especially where in many cases not much calcium carbonate is present. The English greensand marls often contain as much as from 8 to 10 per cent of K_2O, but little or no phosphoric acid.

Review of literature.

During the war the study of practical methods to render the potassium soluble has been stimulated. The Eastern Potash Corporation in New Jersey (13) claims to have found a factory method which consist of treating the greensand with lime and steam under pressure, and a plant is under construction. Ashley (2) deems it entirely feasible to concentrate the glauconite by electric methods, while his experiments with a solution of carbon di-oxid, sulfur di-oxid and dilute hydrochloric acid failed to give results of value for the production of commercial potash. A recently reported study from the Maryland Agriculture Experiment Station (9) shows a possibility of composting greensand with inoculated sulfur and manure as a practical means for farmers to make use of the potassium in the greensand.

A number of experiments have been conducted in Europe in different countries with greensand marls locally found. In America the value of greensand has long been known. In 1819 at least four-fifths of the land in New Jersey around the places were the greensand marl is found, or two-fifths of the whole state was barren according to Morse's American Universal Geography (10). The analyses of the greensand made by Seybert (12) in 1822 shows the existing interest in these early days. A few years later Gordon (6) wrote "It would be difficult to calculate the advatages which the state has gained, and will yet derive from the use of marl. It has already saved some districts from depopulation and increased the inhabitants of others, and may one day contribute to convert the sandy, and pine deserts into regions of agricultural wealth". The prediction of Gordon has come true and much of the land he considered as "deserts" are at present "regions of agricultural wealth". Cook (5) published in 1886 a geological report and gives a large number of analyses of greensand samples. In his discussion ascribes the heavy crops of clover, etc. to the beneficial effects of greensand partly because of the phosphoric acid content and partly because of the sulfuric acid found in it, for "this (sulfuric acid) constituent combines with lime forming plaster" which "efficiency as a fertilizer is well known, though the cause of its action is not satisfactorily explained". Although at that time the part played by the potassium was not well understood, Cook points out that the peculiarly beneficial effect of greensand on potatoes, which contain high amounts of potassium, has strenghtened the opinion that this constituent of the marl is of high value.

The most interesting greensand deposits of New Jersey and Maryland have been described by a number of writers. Clark (4), Ashley (2) and others comment similarly on the value of greensand as a fertilizer. Patterson (11) concludes that the marls of Maryland have very little value for commercial extraction of the potassium on account of the great mass of worthless material in these shell marls. Blair (3) while reporting a number of analyses of New Jersey greensand samples concludes that under the conditions existing in the soil the potash of the greensand marls become gradually available, and since "potash is of especial value to potatoes and to grasses, the benefit derived from marl when used for these crops would lead one to believe that such crops can use the potash of marl to a considerable extent." Lipman and Blair (7) conducted experiments in pots with coarse sand growing barley and buckwheat with a residual crop of soy beans, using greensand as a source of potassium. Barley and buckwheat gave greatly increased yields over the check pots in these experiments. Soy beans fertilized with greensand produced as great a yield of hay as those receiving an application of soluble potassium salts. True and Geise (14)made a study of potassium salts and of greensand in sand cultures, using Shive's complete nutrient solution number R5C2, as a basis. They conclude that "greensand and greensand marls from Virginia and New Jersey are able to supply sufficient potassium to satisfy the demand of Turkey red wheat and red clover during the first two months of their growth, This enables them to make a graeter dry weight of tops then was seen in similar cultures in which the potassium demand was supplied by potassium chlorid, potassium sulfate, and potassium phosphate. Lipman and his coworkers (8) report field experiments with mixtures of greensand marl and inoculated sulfur. The greensand was applied together with acid phosphate and dried blood. The yields obtained with inoculated sulfur alone were as great as the returns from plots receiving a combination of greensand and inoculated sulfur, but greensand alone compared with uninoculated sulfur gave slightly greater yields, but the air dry weights produced by a combination of uninoculated sulfur and greensand was lower than the yields produced by either greensand or sulfur alone or a combination of these two constituents. Ames and Boltz (1) report to have found that the oxidation of the sulfur in the soil as well as dried blood liberated potassium. From these studies they conclude that the liberation of potassium was brought about by the salts rather than by the direct action of the acidity on the insoluble potassium compounds. McCall and Smith (9) composted greensand with sulfur, soil and manure for the purpose of determining the effect of different composts upon the availability of the potassium of greensand. They inoculated with a soil extract known to contain sulfur oxidizing organisms and conclude, after a incubation period of 23 weeks, that "in composts consisting of greensand, manure, and soil in different proportions an appreciable amount of potassium of the greensand was made water soluble". Composts which yielded the largest quantities of water soluble potassium contained the largest proportions of manure, indicating that nitrogen stimulates sulfur oxidizing bacterial activities.

Plan of experiment.

The experiments reported below were conducted primarely for the purpose of determining the effect of inoculated sulfur as compared with uninoculated sulfur upon the availability of potassium. In the second place the object was to study the possibility of decreasing the large quantities of soil used in earlier experiments, so as to make composting of greensand with sulfur more practical for the farmer or manufacturer, and in the third place to determine the effect of ammonium sulfate upon the rapidity with which the potassium in greensand becomes available when no soil is used in the mixtures.

The experiments consisted of composting greensand with sulfur and soil in varying proportions and with additions of ammonium sulfate, Commercial flowers of sulfur were used. The greensand was from Eatontown N.J. with a fairly percentage of potassium and but a trace of calcium. Half of the mixtures were inoculated with infusions known to contain sulfur oxidizing organisms, and to the other half no sulfur oxidizing organisms were added. It was found extremely difficult to keep the uninoculated mixtures free from contamination, and although sterilized sulfur was used much, if not all, of the soil around the New Jersey Experiment Station contains the sulfur oxidizing organisms. The soil was left unsterilized so as not to change the soil flora which would have resulted in placing some of the cultures at a too great disadvantage. The materials were mixed in the following proportions:

Compost number	Composition
1 and 9	100 soil, 20 sulfur, 80 greensand
2 ,, 10	100 ,, ,40 ,, ,60 ,,
3 ,, 11	20 ,, ,20 ,, ,160 ,,
4 ,, 12	20 ,, ,40 ,, ,140 ,,
5 ,, 13	no ,, ,20 ,, ,180 ,,
6 ,, 14	no ,, ,40 ,, ,160 ,,
7 ,, 15	no ,, ,20 ,, ,180 ,, ,0.2 per cent ammonium sulfate
8 ,, 16	no ,, ,40 ,, ,160 ,, ,0.2 per cent ammonium sulfate
9 ,, 18	greensand

Methods.

All materials were air dry and mixed thoroughly before the inoculations were made, and before the ammonium sulfate was added. A part of the mixtures was used for determining the water holding capacity which was made according to the Hilgard method. Water equivalent to 60 per cent of the water holding capacity was than added to the mixtures and they were left standing over night before determinations of hydrogen-ion concentrations, relative acidity, sulfates and potassium were made. The cultures were placed in tumblers covered with glass plates and incubated at 28°C.

Water extractions were made of weighed air dry samples from each compost by shaking 20 grams of mixture with 200 cc. of distilled water in one liter flasks in a shaking machine for 2 hours. The flasks were left standing over night and an aliqaot drawn off for the pH determinations. The remainder of the contents of the flasks were then filtered till the liquid was clear.

The hydrogen-ion concentrations were at first determined according to the method described by Gillespie and later checked up with a portion of the liquid drawn off after the shaking of the mixtures with distilled water. The differences, if any, were always very slight. As indicators the series recommended by Clark and Lubs was used with the apparatus described by Van Alstine (15).

Determinations of relative acidity were made upon aliquots of the water extract. The liquid was boiled to expell carbon di-oxid, cooled, and titrated with 0.1 normal NaOH, using phenolphtalein as indicator.

Potassium determinations were made gravimetrically by the platinic chloric method from aliquots of the water extract, The silicates, iron, aluminium, phosphorus and soluble organic matter were eliminated by evaporation with H_2SO_4, ignition and subsequent precipitation with ammonium hyroxid.

The soluble sulfur was determined by acidifying aliquots of the water extract with concentrated hydrochloric acid and precipitating at the boiling point with barium chlorid. The results are calculated as sulfur tri-oxid (SO_3).

The results recorded are calculated to the moisture free basis of the soil and greensand. Moisture determinations being made by heating portions of air dry material and composts for 15 hours at 105° to 108°C.

The greensand and soil were analysed by the official methods by the beginning of the investiagtion. The results are reported in table 1.

Table 1

Composition of greensand and soil (in per cent)

	Moisture	Insoluble residue	Fe_2O_3 Al_2O_3 MgO Na_2O	K20	P_2O_5	CaO	SO_4	pH
Greensand	3,54	54.11	30.79	4.53	1.16	trace	1.08	6.5
Penn loam	2.17	76.04	12.49	0.49	0.17	0.32	0.52	6.7

From the hydrogen-ion concentration determinations it may be seen that both soil and greensand were slightly acid. The greensand was rather coarse and therefore crushed in a mortar before mixing with the soil. Mechanical analyses of the greensand and Penn loam used, showing their texture, are given in table 2.

Table 2

Mechanical analyses of greesand and soil

Constants	Greensand	Penn loam
	per cent	per cent
Fine gravel	0.42	6.75
Coarse sand	18.64	23.92
Fine sand	42.75	26.25
Very fine sand	29.20	26.56
Silt and clay	8.63	16.32

Experimental results.

Acidity and Hydrogen-ion concentration.

The acidity and hydrogen-ion concentration of the water extracts from each compost were determined at intervals of the inoculated and the uninoculated mixtures. The data obtained are reported in tables 3 and 4.

Accumulation of water soluble acidity and Hydrogen-ion concentrations of inoculated mixtures; acidity expressed in c c. N/10 Na OH required to neutralize acidity of water extract from 10 grams compost; H-ion concentrations expressed in pH values.

No	Composition	After weeks									
		0		1		3		6		7	
		pH	Acidity	pH	Acidity	pH	Acidity	pH	Acidity	pH	Acidity
1	100 soil, 20 sulfur 80 greensand	6.6	0.05	4.5	0.8	2.7	4.0	2.7	11.0	2.2	18.4
2	100 soil, 40 sulfur, 60 greensand	6.6	0.05	4.3	2.9	2.8	8.2	2.2	26.0	2.2	31.2
3	20 soil, 20 sulfur 160 greensand	6.6	0.05	4.6	1.0	2.9	4.5	2.7	8.5	2.4	17.4
4	20 soil, 40 sulfur 140 greensand	6.6	0.05	5.3	0.4	2.9	3.5	2.7	9.5	2.0	19.8
5	20 sulfur, 180 green - sand	6.6	0.05	5.7	0.6	2.9	3.7	2.7	6.65	2.1	16.2
6	40 sulfur, 160 greensand	6.6	0.1	5.9	0.4	3.0	3.0	2.7	7.0	2.4	9.3
7	20 sulfur, 180 greensand, 0.2 percent Ammonium sulfate	6.6	0.1	5.8	1.2	2.9	5.2	3.2	4.7	2.8	10.2
8	40 sulfur, 160 greensand, 0.2 percent Ammonium sulfate	6.6	0.08	5.7	1.3	2.9	5.5	2.6	8.7	2.4	17.2
17	Greensand	6.5	0.02	6.5	0.02	6.2	0.04	6.0	0.1	6.2	0.1

TABLE 3 CONTINUED.

No.	Composition	After weeks					
		9		15		18	
		pH	Acid-ity	pH	Acid-ity	pH	Acid-ity
1	100 soil, 20 sulfur 80 greensand	2.2	30.0	2.3	32.4	2.3	37.6
2	100 soil, 40 sulfur 60 greensand	2.3	33.0	2.2	39.2	2.2	42.0
3	20 soil, 20 sulfur 160 greensand.	2.2	21.5	2.1	24.4	2.2	26.8
4	20 soil, 40 sulfur 140 greensand	2.1	26.5	2.1	30.2	2.0	31.2
5	20 sulfur 180 greensand	2.1	22.0	2.1	27.6	2.0	29.6
6	40 sulfur, 160 greensand	2.5	12.5	2.0	18.4	2.0	25.8
7	20 sulfur, 180 greensand, 0.2 per cent Ammonium sulfate .	2.5	15.5	2.1	20.0	2.1	24.0
8	40 sulfur, 160 greensand, 0.2 percent ammonium sulfate	2.3	23.5	2.0	26.8	2.0	30.0
9	Greensand	5.9	0.1	5.9	0.1	5.9	0.1

TABLE 4.

Accumulation of water soluble acidity and hydrogen-ion concentrations of uninoculated mixtures; acidity expressed in cc N/10 Na OH required to neutralize acidity of water extract from 10 grams compost; hydrogen-ion concentrations expressed in pH values.

No.	Composition	0 weeks		1 week		2 weeks		3 weeks		4 weeks		5 weeks		6 weeks	
		pH	Acidity	pH	acid-ity	pH	Acidity	pH	Acidity	pH	Acidity	pH	Acidity	pH	Acid-ity
9	100 soil, 20 sulfur, 80 greensand.	6.6	.05	5.6	1.0	3.5	2.0	2.9	4.5	2.9	6.0	2.9	8.3	3.0	11.5
10	100 soil, 40 sulfur, 60 greensand.	6.7	.05	4.5	2.1	2.8	4.7	2.8	8.0	2.6	15.5	2.2	18.4	2.4	21.0
11	20 soil, 20 sulfur 160 greensand	6.6	.05	4.8	1.1	3.3	2.3	2.8	5.5	2.8	6.0	2.8	7.6	2.6	9.0
12	20 soil, 40 sulfur, 140 greensand	6.6	.05	5.3	0.8	2.9	1.5	2.9	3.5	2.8	5.5	2.6	6.3	2.6	10.5
13	20 sulfur, 180 greensand	6.6	0.1	5.7	0.3	3.7	0.6	2.9	2.5	2.9	3.0	2.9	3.2	3.1	3.5
14	40 sulfur, 160 greensand	6.6	0.1	5.8	0.4	3.5	1.3	3.0	3.7	2.8	3.5	2.9	3.2	3.2	3.0
15	20 sulfur, 180 greensand, o.2 percent $(NH_4)_2SO_4$	6.6	0.1	5.9	0.2	3.3	2.0	2.9	4.5	2.9	5.0	2.9	3.8	3.3	3.5
16	40 sulfur, 160 greensand, o.2 percent $(NH_4)_2SO_4$	6.6	0.1	5.7	0.3	3.4	2.0	3.0	4.0	2.9	4.0	2.9	3.8	3.1	3.5
18	Greensand	6.5	.02	6.4	.02	6.3	.02	6.2	.03	6.1	.04	6.0	.05	6.0	.07

TABLE 4 CONTINUED.

No.	Composition	7 weeks		9 weeks		15 weeks		18 weeks	
		pH	Acidity	pH	Acidity	pH	Acidity	pH	Acidity
9	100 soil, 20 sulfur 80 greensand	2.9	14.5	2.9	17.5	2.5	23.2	2.2	31.2
10	100 soil, 40 sulfur 60 greensand	2.4	22.2	2.4	26.8	2.2	28.8	2.4	38.2
11	20 soil, 20 sulfur 160 greensand	2.0	14.5	2.2	2.44	2.2	24.4	2.1	24.2
12	20 soil, 40 sulfur 140 greensand	2.1	17.5	2.1	27.5	2.1	29.6	2.1	31.4
13	20 sulfur, 180 greensand	2.9	8.5	2.8	9.4	2.1	11.6	2.3	19.3
14	40 sulfur, 160 greensand	2.5	13.5	2.4	16.8	2.2	17.8	2.3	17.2
15	20 sulfur, 180 greensand, 0.2 percent $(NH_4)_2SO_4$	2.6	15.0	2.4	18.6	2.2	25.6	2.2	27.0
16	40 sulfur, 160 greensand, 0.2 percent $(NH_4)_2SO_4$	2.6	13.0	2.4	18.2	2.2	20.2	2.3	22.8
18	Greensand	6.2	.08	5.9	.1	5.8	.1	5.9	.1

It is clear from the data presented that no great differences were fpund between the cultures with inoculated and uninoculated sulfur. As has been pointed out this was to be expected since it is extremely difficult to keep the uninoculated mixtures from contamiation under ordinary laboratory conditions. All cultures show a rapid change in H-ion concentration and a gradual increase in titratable acidity. After 6 weeks one of the cultures to which soil was added had reached the point of hydrogen-ion concentration at which the potassium of the greensand becomes available, which point was reached by most of the cultures after an incubation period of 9 weeks. From then on the H-ion concentration changed but slightly, although acidity accumulation went on, showing that sulfur oxidation proceeded. These slight changes in H-ion concentration indicate that but relativily small amounts of free acid existed and nearly all of the acidity titrated existed in the form of sulfates. The mixtures with soil in addition made from the beginning somewhat larger quanitities of acidity, but the intensity of the acid produced was generally less than in the cultures without soil. The quantities of acidity titrated were highest in the mixtures with 50 per cent soil. Addition of ammonium sulfate did not seem to have much influence upon the quantity of acid produced, nor upon the intensity of the acid produced, except some possible slight differences in the uninoculated cultures between those receiving neither soil nor ammonium sulfate and those receiving ammonium sulfate but without soil. There seemed to be sufficient nitrogen available for the needs of the organisms to last untill the end of the experiment.

Sulfate formation.

The production of water soluble sulfates during the first weeks of incubation was very rapid in all mixtures. If the results recorded in tables 5 and 6 are compared it may be seen that after an incubation period of 15 weeks the inoculated mixtures had produced more sulfates than the uninoculated composts.

TABLE 5

Accumulation of water soluble sulfate of inoculated composts, expressed in milligrams, sulfur tri-oxid per 100 grams of mixture.

No.	Composition	After weeks						
		0	1	3	6	9	15	18
1	100 soil, 20 sulfur, 80 greensand	1.32	14.19	63.03	71.27	200.24	218.99	233.08
2	100 soil, 40 sulfur, 60 greensand	1.30	16.62	74.32	116.88	186.74	211.25	228.66
3	20 soil, 20 sulfur 160 greensand	1.48	14.88	65.99	93.11	158.22	184.96	200.85
4	20 soil, 40 sulfur, 140 greensand	1.48	12.22	54.78	90.97	184.99	204.89	206.92
5	20 sulfur, 180 greensand	1.70	15.30	64.00	88.29	161.16	207.47	212.07
6	40 sulfur, 160 greensand	1.68	13.20	59.14	90.24	114.58	164.81	184.47
7	20 sulfur, 180 greensand, 0.2 percent $(NH_4)_2SO_4$	3.72	17.84	79.72	88.01	147.50	184.60	198.89
8	40 sulfur, 160 greensand, 0.2 percent $(NH_4)_2SO_4$	3.40	16.96	66.30	101.76	150.18	193.92	215.27
17	Greensand	1.08	1.18	1.60	6.23	6.60	9.70	9.52

Accumulation of water soluble sulfate of uninoculated mixtures, expressed in milligrams sulfur tri-oxid per 10 grams compost.

No.	Compositions	After weeks						
		0	1	3	6	9	15	18
9	100 soil, 20 sulfur, 80 greensand .	1.32	13.10	58.62	70.45	101.66	145.44	197.86
10	100 soil, 40 sulfur 60 greensand.	1.30	15.96	71.38	128.85	163.74	192.35	218.15
11	20 soil, 20 sulfur, 160 greensand	1.48	16.26	73.32	102.07	146.92	161.99	179.14
12	20 soil, 40 sulfur, 140 greensand .	1.48	13.36	59.78	102.13	173.01	184.43	199.92
13	20 sulfur, 180 greensand	1.70	12.38	55.36	86.14	101.02	141.54	170.77
14	40 sulfur, 160 greensand	1.68	12.06	33.58	50.83	113.85	159.17	149.55
15	20 sulfur, 180 greensand, 0.2 % $(NH_4)_2SO4$	3.72	16.81	75.16	83.94	142.19	158.52	192.40
16	40 sulfur, 160 greensand, 0.2 % $(NH_4)_2SO_4$.	3.40	15.22	67.51	71.22	130.95	182.62	190.86
18	Greensand	1.08	1.26	1.73	7.68	6.58	9.99	9.83

This gain, however, was largely lost after 18 weeks, due to the fact that the sulfate accumulation was slow between the 9th and 18th weeks of incubation. The reason for the slower accumulation may possibly be that the sulfates and free acid formed at first do not react with the mineral constituents present, or do so only very slightly, but when sufficient amounts are produced a comparativily rapid reaction takes place with the extremely small particles, while later on a slower reaction takes place with the more coarse material. This would indicate that finely ground greensand would yield greater amounts of the water soluble potassium.

Water soluble potassium.

The increase in water soluble potassium was gradual in all cultures as is shown by the data recorded in table 7 and 8.

TABLE 7

Accumulation of water soluble potassium of inoculated mixtures, expressed in milligrams per 10 grams of compost.

No.	Composition	After weeks						
		0	1	3	6	9	15	18
1	100 soil, 20 sulfur, 80 greensand	1.24	3.84	5.27	5.64	6.16	7.13	7.91
2	100 soil, 40 sulfur 60 greensand	1.08	2.09	4.03	5.38	6.48	6.32	7.39
3	20 soil, 20 sulfur, 160 greensand	0.72	2.76	2.95	3.43	7.72	9.43	11.79
4	20 soil, 40 sulfur 140 greensand	0.56	3.81	4.71	5.43	7.75	8.98	12.68
5	20 sulfur, 180 greensand.	0.62	2.72	2.74	3.01	8.52	12.08	16.48
6	40 sulfur, 160 greensand	0.53	2.83	2.97	3.53	7.79	9.02	11.47
7	20 sulfur, 180 greensand, 0.2 percent Ammonium sulfate	0.36	2.04	2.14	2.84	8.37	9.44	12.71
8	40 sulfur, 160 greensand, 0.2 per cent Ammonium sulfate	0.20	2.44	2.80	2.90	8.79	9.63	11.71
17	Greensand	0.25	0.29	0.47	0.41	0.41	0.41	0.29

TABLE 8

Accumulation of water soluble potassium of uninoculated mixtures, expressed in milligrams per 10 grams of compost.

No.	Compositions	After weeks						
		0	1	3	6	9	15	18
9	100 soil, 20 sulfur, 80 greensand.	0.54	1.86	2.49	4.51	4.20	5.94	7.23
10	100 soil, 40 sulfur 60 greensand	0.82	1.16	1.83	4.47	4.53	5.42	6.07
11	20 soil, 20 sulfur 160 greensand	0.27	0.56	1.07	3.88	7.39	9.48	11.07
12	20 soil, 40 sulfur 140 greensand.	0.45	0.73	1.18	2.73	4.67	8.98	11.31
13	20 sulfur, 180 greensand	0.15	0.46	1.87	1.87	7.59	7.06	12.27
14	40 sulfur, 160 greensand	0.27	0.79	1.79	2.86	4.08	6.09	11.31
15	20 sulfur, 180 greensand, 0.2 percent Ammonium sulfate	0.18	0.68	1.14	3.84	4.76	6.11	13.80
16	40 sulfur, 160 greensand, 0.2 percent Ammonium sulfate.	0.18	0.41	1.22	3.26	4.72	6.42	12.18
18	Greensand	0.25	0.24	0.41	0.41	0.38	0.29	0.36

Untill the 15th week the inoculated cultures were ahead of the uninoculated,but after 18 weeks not much difference was apparent. The possible reason has been pointed out by the discussion of the accumulation of water soluble sulfates.

The total water soluble potassium seemed to be greatest in all mixtures without soil additions,but if calculated on the basis of the per cent of total potassium present this is reversed.

It is necessary that a certain degree of acidity be produced before the potassium becomes water soluble. The amounts of sulfates formed do not necessarily have to reach a certain quantity,but the acidity produced has to be of a certain intensity. The point at which the potassium of the greensand becomes more rapidly water soluble lies between the pH values 2.7 and 2.3 as is shown by determinations made from greensand extracts treated with diluted sulfuric acid. Nevertheless,the quantities of sulfates formed have a close relation to the intensity of the free acid formed.

The curves in fig.1,show clearly the relation between hydrogen-ion concentration,acidity accumulation,sulfate formation and water soluble potassium in two of the inoculated mixtures.

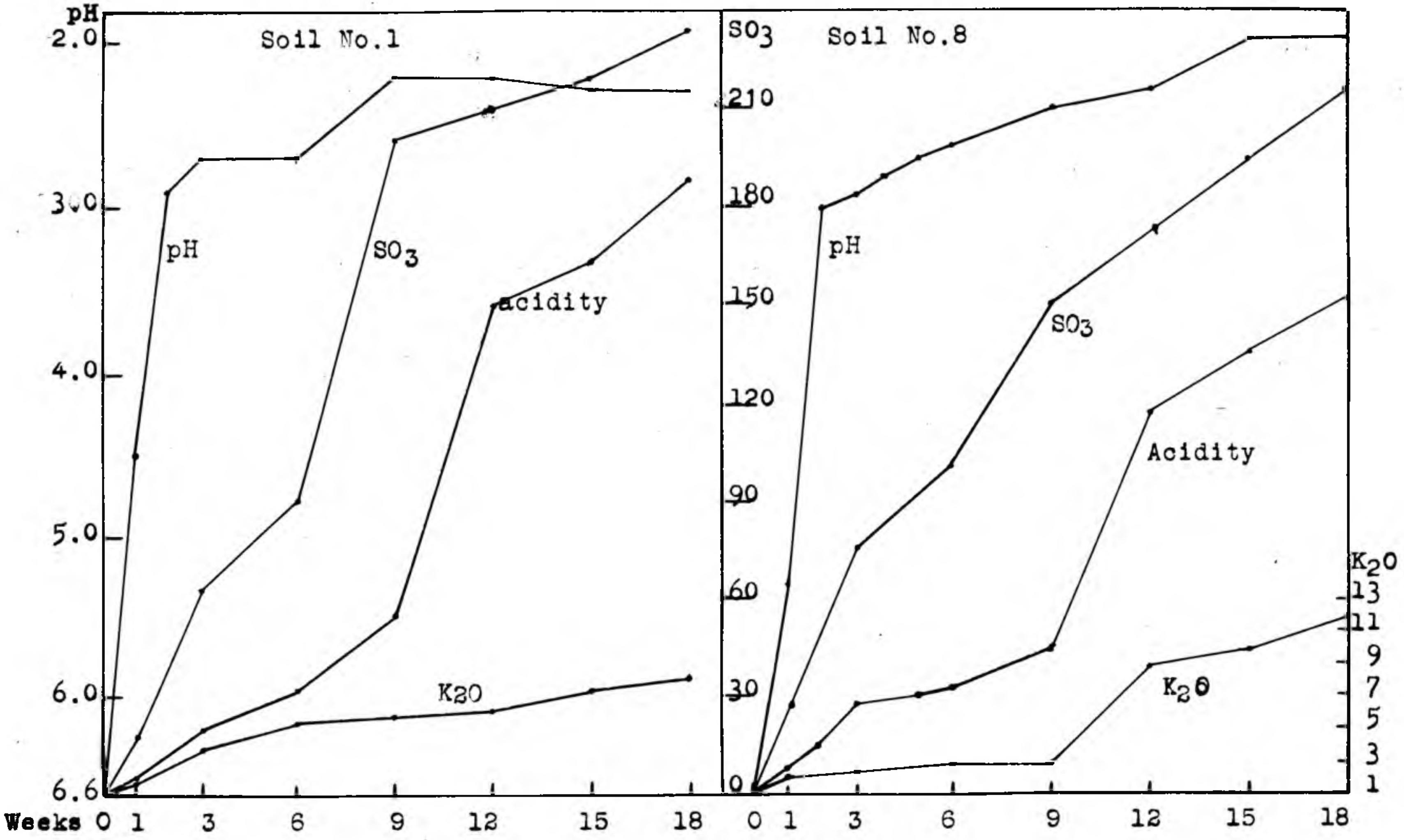

Fig.I. Graphs showing the relation between hydrogen-ion concentration, sulfates, acidity and soluble potassium in compost mixtures numbers1 and 8.

It seems evident that a part of the acid or acid sulfates formed in culture number 1 reacted with the soil constituents,while in culture number 8 these sulfates or the free acid acted more directly upon the potassium of the greensand. It would seem that most rapid and thus most economical results would be obtained if a part of the composts were used for inoculation of new compost mixtures, eliminating thereby the long period of incubation before a certain hydrogen-ion concentration and a certain acidity accumulation is reached.

As has been pointed out above the total water soluble potassium seemed to be greatest in all mixtures containing no soil,but from the data presented in table 9 it is evident that a smaller percentage of the total potassium present had been liberated in the cultures without additions of soil. It should be kept in mind that the greensand contained but 4.63 per cent of total potassium and much of the materials present reacts with the acid or acid compounds formed. Besides,the reaction was not complete and still continuing at the end of 18 weeks. Since only a small amount of the sulfure was oxidized it does not seem necessary to add such large quantities of sulfur as was done in this experiment. The cultures to which potassium obtained.

Culture Number	Sulfur in mixture (grams)	Sulfur oxidized in grams	Percent Sulfur oxidized	Potassium in mixture (gr.K_2O)	Soluble Potassium	Percent soluble potassium
1	20	1.864	9.32	3.624	0.1582	4.37
2	40	1.830	4.57	2.718	0.1478	5.44
3	20	1.606	8.03	7.248	0.2358	3.26
4	40	1.658	4.14	6.342	0.2536	3.99
5	20	1.698	8.49	8.154	0.3296	4.04
6	40	1.474	3.68	7.248	0.2294	3.17
7	20	1.592	7.96	8.154	0.2542	3.12
8	40	1.723	4.31	7.248	0.2342	3.23
9	20	1.578	7.89	3.624	0.1446	3.92
10	40	1.746	4.36	2.718	0.1214	4.47
11	20	1.442	7.21	7.248	0.2214	3.07
12	40	1.588	3.97	6.342	0.2262	3.57
13	20	1.369	6.85	8.154	0.2454	3.01
14	40	1.196	2.99	7.248	0.2262	3.13
15	20	1.540	7.70	8.154	0.2760	3.38
16	40	1.528	3.82	7.248	0.2435	3.36
17	None			9.06	0.0007	0.078
18	"			9.06	0.0006	0.067

40 grams of sulfur were added had accumulated almost exactly the same amounts of sulfates as the cultures to which 20 grams of sulfur were added. The relation between the per cent of potassium liberated and the per cent of sulfur oxidized was roughly 1:2. It seems therefore, that approximately 10 per cent of sulfur of the total greensand is sufficient to secure satisfactory yields.

Sand cultures.

The per cent of water soluble potassium liberated was low in all cases, but it seemed sufficient to supply plants with the necessary amounts of potassium. In order to test whether or not the available potassium would be sufficient to support plants with this necessary plant food element, pot cultures were conducted with soy bean plants. Since, as has been shown by earlier investigations, potassium absorption is greatest during the first part of the growing period of the plants, soy beans were grown for only 6 weeks. Soy bean plants ordinarily bloom in the greenhouse between the 4th and 5th week and the growing period of 6 weeks would be enough.to test the value of the material for these plants.

Although it is known that the ordinary glased earthen ware pots furnish very small amounts of soluble impurities amoung which potassium compounds, it was thought that the differences among the cultures would be large enough to gave this test practical value. As a source of the necessary nutrient salts Shive's cultural solution R_5C_2 was used,

To 5 kilograms washed quartz sand were added the following constituents:

1. None
2. Greensand (dry)
3. Shives cultural solution R_5C_2
4. Composted greensand
5. Shive's solution, except potassium, plus greensand (dry)
6. Shive's solution, except potassium, plus composted greensand.

The greensand substituted for the potassium in Shive's cultural solution was calculated to be approximately that necessary to supply an amount of potassium aquivalent to that in the solution. The inoculated greensand had been composted for a period of 18 weeks previous to the application, The results from the plants grown in these cultures would thus be comparable with the results of the plants grown in cultures with the easily available potassium of Shive's cultural solution.

The soy bean seeds were selected for size and germinated in sand. The seedlings were then selected again to be as nearly alike as possible and planted when about two inches high. After the planting the cultures were inoculated with a few cc. of a water extract known to contain nodule forming bacteria. The yields obtained after a grewing period of 6 weeks are presented in table 10 in the form of dry weights of tops, lenght of plants, numbers of pods produced, and the pH values at the beginning and at the end of the experiment.

Table 10

Yields of tops of soy beans grown in sand cultures, with Shive's nutrient solution as a basis, whereby the potassium of the solution is replaced by the potassium of composted greensand.

Culture number	Additions	Dry weights of tops in grm.	Average height in cm.	Number of pods	pH begin	pH end	Nodules
1	none	0.856	8	bloom	5.7	6.8	small
11	greensand	1.132	9	2	5.3	6.1	large
4	R_5C_2	3.356	14	4	5.4	6.4	few
10	composted greensand	1.206	8.5	2	5.4	5.8	abundance
13	R_5C_2, greensand	2.501	13	6	5.0	6.5	few, but large
12	R_5C_2, composted greensand	3.299	14	9	4,8	6.4	not many but large

It will be noted that the greensand (dry) alone had some influence on the growth of these soy bean plants.

The dry greensand substituted for the potassium in the cultural solution was apparently not able to replace the potassium fully for the needs of these plants in this period of growth, but the composted greensand seemed to render sufficient potassium available for their needs. In fact, the cultures receiving greensand as a substitute matured earlier and seemed more vigorous than the plants in Shive's nutrient solution.

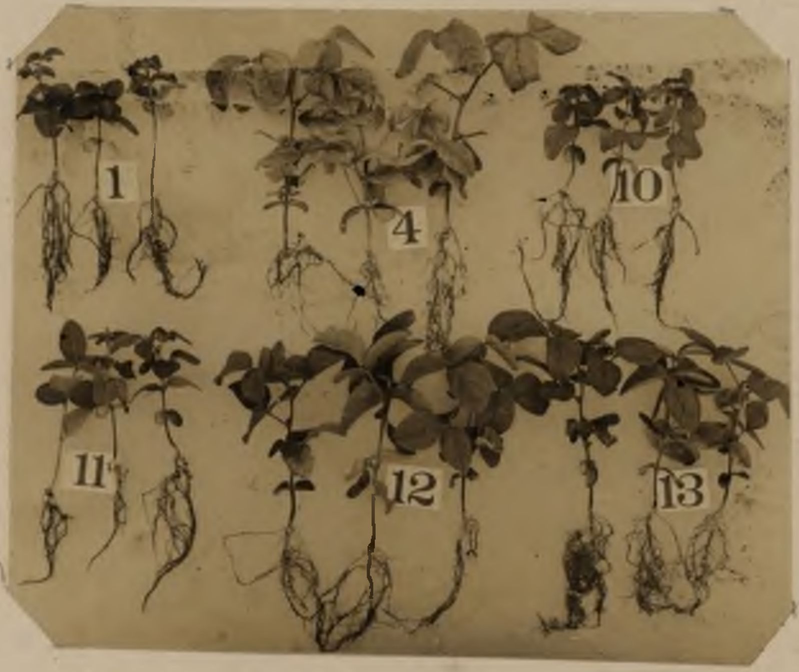

Fig.2. Soy bean plants grown in sand cultures, with additions of greensand.

From the numbers of pods produced it can be seen that the plants receiving greensand as a substitute for the potassium in Shive's solution would yield also more seeds, showing that their earlier maturity was profitable instead of detremental, although slightly less hay was produced as compared with the plants grown in the R_5C_2 solution. In addition, more and larger nodules were noticed on the roots of all plants receiving potassium in the form of greensand.

CONCLUSIONS.

1. In composts consisting of greensand and sulfur small amounts of potassium are liberated.
2. No great differences occured between inoculated and uninoculated sulfur-greensand mixtures, because there was contamination of the uninoculated mixtures.
3. The potassium of the grensand id made available at a certain hydrogen-ion concentration, lying between the pH values 2.7 and 2.3.
4. There is a definite relation between the hydrogen-ion concentration and sulfate formation, and the water soluble potassium.

5. Soy bean plants grown in quartz sand and with Shive's nutrient solution inwhich composted greensand was substituted for the potassium of the cultural solution are able to make as great a dry weight of tops as in similar cultures in which the potassium was supplied in the form of potassium phosphate. Soy bean plants receiving composted greensand as a source of potassium matured earlier and yielded more seeds than the plants grown in Shive's cultural solution.

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PART FOUR.

SULFUR OXIDATION IN HANFORD LOAM AND FRESNO "BLACK ALKALI" SOILS FROM CALIFORNIA.

The oxidation of sulfur in the soil, and the influence of sulfur upon bacterial activities and development has been studied by many investigators. Sulfur transformation in the soil has long been known, but only in recent years has the mecanism of it been more fully under consideration.

Although no attempt is made to give a complete bibliography on the subject, some of the more conclusive studies reported are included in the following revue of literature.

Boulanger and Dugardin (3), in trying to explain the fertilizing action of sulfur, found that the effect of sulfur on crop yields was more marked with unsterilized soil than with sterilized soil, due to the oxidation of sulfur by bacterial activities. Demolon (8) studying the fertilizing action of sulfur found that but little sulfur was oxidized in a sterilized garden soil, far greater amounts being oxidized when the soil was left unsterilized. Bernhard (2) states that the beneficial effect of sulfur for the comtrol of potato scab, was due to the disinfection of the soil by sulfur. Brown and Kellogg (5) showed that different soils have unlike "sulfofying powers" and some of the factors influencing the change of elemental sulfur to the sulfate form were of a biological nature. Lipman and his associates (17) have shown that elemental sulfur is oxidized by the proper bacteria. Later, a number of studies from the New Jersey Experiment Station laboratory, have confined the earlier experiments. Martin (19) publiced recently the results obtained with inoculated and uninoculated sulfur for the control of potato scab, which show that inoculated sulfur produced greater amounts of soil acidity then did uninoculated sulfur, and was superior for the control of potato scab.

There has been, and still is, considerable contraversy as to the necessity of applying sulfur to the soil for the stimulation of plant growth. Lyon and Bizzel (18) report that in the lysimeter experiments at Cornell the sulfate sulfur in the drainage water was from three to six times as great as in the crops, while the sulfur content of the drainage water from the unplanted soil was about equal to the sulfur content of the crops and drainage water from the planted soil. Swanson and Miller (28) state that the loss of sulfur due to the amount taking up by crop is insignificant as compared with the total amount which has disappeared from the soil. Hart and Peterson (10) calculate the loss of sulfur in drainage water to be three times the amount brought down to an acre from the atmosphere. Stewart (27) concludes that under humid conditions sulfur need not be added to the soil as plant food. Sufficient sulfur is brought down by the rainfall to supply plant needs. Ames and Boltz (1) conclude that the cultivation of silt loam for 16 years without the addition of fertilizers has decreased the total sulfur supply.

Some striking results have been obtained with the use of sulfur as a fertilizer or stimulant. Reimer (24) obtained large increases in alfalfa yields by the use of elemental sulfur. A number of Oregon soils are deficient in sulfur and respond readily to sulfur applications as is shown by the work of Reimer and Tartar (25),who report increases of from 50 to 1000 per cent for alfalfa and clover by the use of fertilizers containing sulfur on various soil types. Flowers of sulfur produced beneficial results similar to those obtained by the use of super-fosfate,gypsum,iron-sulfate,etc. Headley (11) reports experiments with sulfuric acid and gypsum and states that the yields of the soils so treated has been decidedly greater each year than those from the untreated plots,but not enough greater to be profitable. Miller (20) concludes from pot experiments that the addition of elemental sulfur enhanced the growth of the plants,and that the corresponding increases obtained on the soil extract indicated that sulfur acted directly in promoting this growth. Tottingham and Hart (30) made greenhouse and field tests with sulfur and composted sulfur and rock phosphate. They conclude that on two loam soils sulfur increased the growth of clover and crusiferae on one soil and not on the other. Sulfur increased the yields of abrley seed on a silt loam apparantly in need of lime. One hundred pounds of sulfur per acre was most effective.

The action of sulfur and the sulfates formed in the soil upon the soil bacteria and their actvities has been studied in several instances. Janicaud (14) states that sulfur has a favorable influence on the development of bacteria in the soil. Duley (9) concludes that sulfur and gypsum increase the number of nodules to a marked extent on red clover roots in certain Missouri soils. Boulanger and Dugardin (3) studied the effect of sulfur on ammonification,nitrofication,and nitrogen fixation and they found that the presence of small amounts of sulfur decidedly increased the activity of ammonifying bacteria. Brioux and Geurbet (4) challenged the conclusions of these investigators and say that sulfur as such does not have any influence upon the activities of ammonifying or nitrofying bacteria. O'Gara (21) reporting field experiments with field and truck crops in which elemental sulfur was added at a rate of 400 pouds per acre states that " one of the striking effects of sulfur treatment of soils on the soil microorganisms is the decided and consistent increase in total bacterial numbers as determined by the plate method." Tacheuchi (29) concludes that sulfur applied at the rate of 0.5 per cent and over decreases the number of bacteria in the soil, and that sulfur alone does not affect the root development, or the number of root nodules.

Miller (20) states that the great increase in the nitrogen content of clover grown on soil where sulfates had been added is the result, in all probability, of the sulfates stimulating the action of the legume bacteria; the number of nodules on the clover roots were increased. Pitz (23) found elemental sulfur to increase soil acidity, and that the number of bacteria which would grow on agar plates decreased after a certain period, if sulfur was added to a silt loam soil. He reports an increase inammonification, accompanied by a parallel decrease in nitrate formation.

A. Experiments with slight alkaline soils.

From the short review of literature it seems clear that some soils do respond to the treatment of sulfur and others do not. The study reported below was primarely undertaken to determine whether or not a slight alkaline soil, which produces chlorosis, would be benefitted by additions of sulfur.

Determinations were made on numbers of the microorganisms in this soil, influence on the physical structure (flocculation, turbidity, water holding capacity, change in volume), and the influence on the growth of soybeans.

The soil used was a Hanford fine sandy loam, and secured by Prof. C. F. Shaw of the University of California from ~~a~~ ranches near Pomona, Cal. Prof. Shaw wrote the following notes about these soils and their history:

"These soils were secured from a point about two miles west of the business center of Pomona. Soil No. 1 was taken in an orange grove about 100 feet from the west line of the orchard. The ornge trees were about thirty years old. They have been manured annually with barnyard manure, but the quantity applied was unknown by the owner. He has approximately seven acres in the grove and applies to it all the manure from one horse and one cow, spreading it on as it is made. The grove has been irrigated by furrows ever since it was first set out. The water has flowed from the east towards the west and the block of soil between each row has not received water except as it has run over from the shallow furrows or has seeped in by lateral movement. There has been slight accumulation of finer sediments in the west side of the grove ---the side from which the soil was taken. The soil was taken from about the middle of the row between the trees and from the low ridge between two irrigation furrows. The land had been irrigated about one week previous. These ~~soils~~ Samples represent a good soil in which the fertility has been maintained reasonably well thruogh fertilizing and through tillage.

"Soil number 2 was taken about 150 feet west of number 1 and about 50 feet west of the boundray line between the orange grove and the adjoining corn field. The field from which soil number 2 was taken has been in corn or in barley annually for a great many years. It has been farmed nearly every year for thirty or more years,and no fertilizer has ever been applied,and the system of culture is poor,the ranch being run by a Mexican tenant.During the last 6 years the field has grown at least 4 and possibly 5 crops of corn and one and possibly 2 crops of barley. At first the crop yields were good but they have dropped off during the last ten years. The field was this year in corn with a fair stand."

X mechanical analyses made of these soils showed that they were very fine. The data is given in table 1.

Table 1

Machanical analyses of Hanford fine Sandy Loam

Soil	Orange grove	Open field
Fine gravel	per cent 2.85	per cent 6.65
Coarse sand	9.99	9.34
Fine sand	11.02	11.21
Very fine sand	33.58	33.05
Silt and clay	42.56	38.91

The chemical analyses given in table 2 indicate that these soils were poor in nitrogenous matter:

Table 2

Chemical analyses of Hanford fine Sandy loam

	Orange grove per cent	Open field per cent
CaO, MgO, Fe_2O_3	0.3175	0.2805
K_2O	0.565	0.633
P_2O_5	0.1033	0.0877
N (total)	0.0665	0.0593
SO_4	0.0107	0.0148
Cl	0.0089	0.0111
Total salts (water soluble	0.3821	0.1991
Total alkalinity)	0.21	0.22
pH	7.2	7.1

) Expressed in 10/N H_2SO_4 per 10 grams of soil.

The flowers of sulfur were inocculated with one per cent of soil which was known to contain sulfur oxidizing organisms. The sulfur was thoroughly mixed with the soil and the water holding capacity determined according to the Hilgard (12) method. The soils were kept at 60 per cent of the water holding capacity throughout the period of ivestigation.

Hydrogen-ion and hydroxyl-ion concentration determinations were made of the soil before and after mixing with sulfur and at definit intervals during the growth of the plants, using the apparatus decribed by Van Alstine (30).

The soils were divided into two parts, one to be used for plant cultures and the other for bacterial studies.

Experimental results.

SeriesI. A. Vegetation experiments with soil number 1.

Soy beans were grown in earthenware pots filled 5 kilogram of soil. Different amounts of inoculated sulfur were thoroughly mixed with the soil. To duplicates were added, besides the same quantities of inoculated sulfur, 300 pounds of rock phosphate per acre, and to triplicates, in the place of rock phosphate, 100 pounds of acid phosphate per acre were added. The exact amounts of sulfur added to all series were as follows:

Culture number	Pounds of sulfur per acre
1,6,11	none
2,7,12	100
3,8,13	300
4,9,14	500
5,10,15	1000

The soy bean seeds were selected for size, germinated, and transplanted when from 2 to 3 inches high, care being taken to select plants as nearly alike as possible. Six plants were planted in each pot and grown for 9 weeks. At the beginning of the experiment the soil was inoculated with a water extract of a soil containing soy bean nodule forming bacteria.

The optimum water content of the soil was maintained by daily additions of distilled water the pots being placed on the scale pan every 2 or 3 days.

Notes were taken at intervals and the hydrogen-ion concentration of the soil extract determined once each week.

The changes of hydrogen-ion exponents during the growing period are recorded in table 3.

SERIES I **TABLE 3**

Hydrogen-ion concentrations, expressed as pH values, of soil water extracts before and during the growth of soybean plants.

		After weeks;									
No.	Sulfur added in pounds per acre	0	1	2	3	4	5	6	7	8	9
1	None	7.0	6.9	7.0	7.2	7.0	6.8	7.0	6.9	6.9	6.9
2	100	7.0	6.9	6.9	6.7	6.9	6.7	7.0	6.9	6.9	6.9
3	300	7.0	7.0	6.8	6.5	6.6	6.6	6.9	6.9	6.8	6.6
4	500	6.9	6.8	6.9	6.7	6.6	6.4	6.9	6.8	6.7	6.6
5	1000	6.9	6.8	6.9	6.7	6.7	6.5	6.6	6.6	6.4	6.2
		In addition to sulfur 300 pounds of rock phosphate									
6	None	7.0	7.0	6.9	6.7	6.8	6.7	6.9	6.9	6.9	6.9
7	100	7.0	7.0	6.9	6.6	6.6	6.6	6.9	6.9	6.8	6.8
8	300	7.0	6.9	7.0	6.6	6.6	6.4	6.7	6.7	6.6	6.5
9	500	6.9	7.0	6.9	6.5	6.4	6.5	6.5	6.6	6.3	6.1
10	1000	6.9	6.7	6.8	6.7	6.6	6.4	6.6	6.6	6.3	6.2
		In addition to sulfur 100 pounds of acid phosphate									
11	None	7.0	6.9	7.0	6.8	6.7	6.5	7.1	7.0	7.0	7.0
12	100	7.1	6.9	6.9	6.7	6.7	6.6	6.9	6.9	6.8	6.8
13	300	7.0	7.0	6.9	6.6	6.6	6.4	6.8	6.9	6.7	6.6
14	500	7.0	6.9	6.7	6.7	6.6	6.2	6.7	6.6	6.4	6.2
15	1000	7.0	6.7	6.7	6.6	6.6	6.5	6.6	6.5	6.3	6.2

The influence of plant growth on the hydrogen-ion concentration was but slight. In the water extract of the soils traeted with small amounts of sulfur the changes were not very marked, but the larger quantities of sulfur exerted a graeter influence, although not as much as could be expected from the rather heavy applications. This was probably due to the slow oxidation of the sulfur.

Notes taken after 3 weeks show that, in general, the plants in soil to which sulfur was added were slightly behind the check cultures. Most of these plants started to develop small yellow spots on the leaves. According to the notes taken after 6 weeks, when 3 of the 6 plants were harvested, it appears that these yellow discolorations, which more or less resembled mosaic, were not found on the plants grown in soils which received 300, 500 and 1000 pounds of sulfur per acre, and that the plants grown in soil to which the same sulfur addition and rock phosphate were made, had been but very slightly affected, while the affect on the plants grown in the cultures receiving the same amounts of sulfur and acid phosphate in addition, was more distinct. The yellow spots on the plants in the other cultures were at that time more pronounced than after 3 weeks.

A comparison made at the end of 6 weeks of all pot-cultures seemed to place the plants receiving sulfur alone as best.

After 9 weeks the plants were scored again and the yellowish, sickly looking leaves, counted. In all cases the yellowness increased with the increase of the quantities of sulfur employed. The plants grown in soil with sulfur alone seemed to be still ahead. At the end of 9 weeks the plants were harvested, and a comparison made on the relative numbers of nodules and on the extent of the root system. An idea of the plants grown in soil receiving sulfur alone may be had from fig.1, taken just before harvesting.

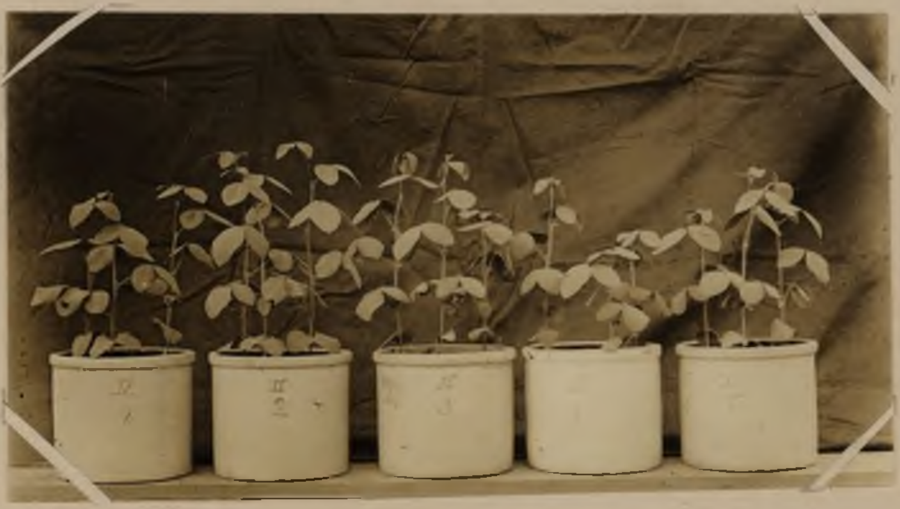


Fig.1. Soy beans receiving sulfur alone.

It appeared that the check plants had all a more extensive root system than the plants grown in the sulfur treated soils, with the exception of plants receiving 100 pounds of sulfur to the acre. On the latter the nodules were also more numerous than on the check plants and the plants treated with higher amounts of sulfur. The nodules decreased numerically with the increase of the amounts of sulfur applied. The plants which were grown in the soil receiving 1000 pounds of sulfur per acre had but very few extremely large nodules. Some of these nodules were from ¾ to 1 centimeter in diameter.

The combined dry weights of the soy bean tops, together with total transpiration (plus evaporation), avarage height in centimeters, pH values at begin and at the end of the experiment, and the number of pods produced, are given in condenced form in table 4.

TABLE 4

Yields of tops of soybeans grown in orange grove soil for 9 weeks.

No.	Dry weight in grams	Relative weight none = 100	Transpiration in cc.	Average height in c.m.	Number of pods	pH begin	pH end	Remarks.
1	4.2656	100.0	3482	25.2	5	7.2	6.9	Many small pods.
2	4.4153	103.5	3605	31.2	7	7.2	6.9	
3	4.2865	100.8	3573	28.7	8	7.2	6.6	Earlier mature.
4	3.6462	85.6	3384	22.4	2	6.9	6.6	10 small pods.
5	3.9507	92.7	3377	25.6	5	6.9	6.2	number small pods.
			In addition to sulfur 300 pounds rock phosphate					
6	3.8648	100.0	3431	25.6	10	7.0	6.9	
7	3.9702	103.9	3366	28.0	9	7.0	6.9	
8	4.5278	117.2	3388	24.0	3	7.0	6.8	2 plants blooming, least mature (green)
9	3.7750	97.7	3283	25.6	7	7.1	6.5	
10	3.3295	86.4	3326	26.4	4 (poor)	6.9	6.1	most mature (yellow)
			In addition to sulfur 100 pounds of acid phosphate.					
11	4.3178	100.0	3517	24.0	5	7.0	7.0	
112	3.7555	86.9	3284	21.6	blooming	7.1	6.8	darkest green
13	4.1457	95.9	3497	25.6	6	7.1	6.6	
14	4.0799	94.6	3580	27.2	4	7.0	6.2	
15	3.7872	87.6	3525	26.4	blooming	7.1	6.2	

It is evident from table 4 that, although the weight of the plants decreased towards the highest sulfur application, the differences were not striking. It was concluded therefore, to grow another crop of soy beans in the same pots with similar applications of sulfur, rock phosphate and acid phosphate.

The plants were selected and planted as before and three out of the six plants harvested after 4 weeks, leaving the 3 best looking plants in each pot. At this time the cotolydons of the check plants and of the plants receiving 1000 pounds of sulfur were all still dark green, while most of the cotolydons of the plants receiving 100, 300 and 500 pounds of sulfur per acre, had been dropped previously or had turned yellow before the end of 4 weeks. The best looking plants were at that time in the check pots.

The changes in hydrogen-ion concentrations during the growth period are recorded in table 5.

Hydrogen-ion concentrations, expressed in pH values, of soil water extracts before and during the growth of the second crop soybeans.

No.	Sulfur added in pounds per acre	After weeks; 0	1	2	3	4	5	6
1	None	6.9	7.0	6.9	7.0	6.9	6.9	6.6
2	100	6.9	6.9	6.9	7.0	6.9	6.8	6.5
3	300	6.6	6.7	6.7	6.9	6.8	6.5	6.3
4	500	6.6	6.6	6.6	6.7	6.7	6.3	6.1
5	1000	6.2	6.5	6.6	6.6	6.3	6.1	6.0
			In addition to sulfur 300 pounds of rock phosphate					
6	None	7.0	6.9	6.8	6.9	6.9	6.9	6.7
7	100	6.9	6.9	6.9	6.9	6.9	6.8	6.4
8	300	6.8	6.6	6.6	6.7	6.7	6.4	6.2
9	500	6.5	6.5	6.5	6.3	6.5	6.1	6.1
10	1000	6.1	6.1	6.2	6.1	5.6	4.6	4.6
			In addition to sulfur 100 pounds of acid phosphate.					
11	None	7.0	6.9	6.8	7.0	6.9	6.9	6.7
12	100	6.9	6.8	6.9	6.9	6.8	6.8	6.5
13	300	6.8	6.6	6.9	6.9	6.7	6.5	6.3
14	500	6.6	6.5	6.9	6.7	6.3	6.2	6.1
15	1000	6.2	6.2	6.3	6.3	6.2	6.0	5.8

It is interesting to note that the pots receiving 1000 pounds of sulfur per acre and 300 pounds of rock phosphate in addition had a much higher hydrogen-ion concentration (lower pH values) than the pots receiving acid phosphate in addition, and also higher than the pots which received 1000 pounds of sulfur alone. The H-ion concentration went up more or less in all cultures.

According to the noted taken all plants were found blooming after four and one half weeks, while culture numbers 5,10 and 15 had very yellow leaves of which some had been dropped at that time.

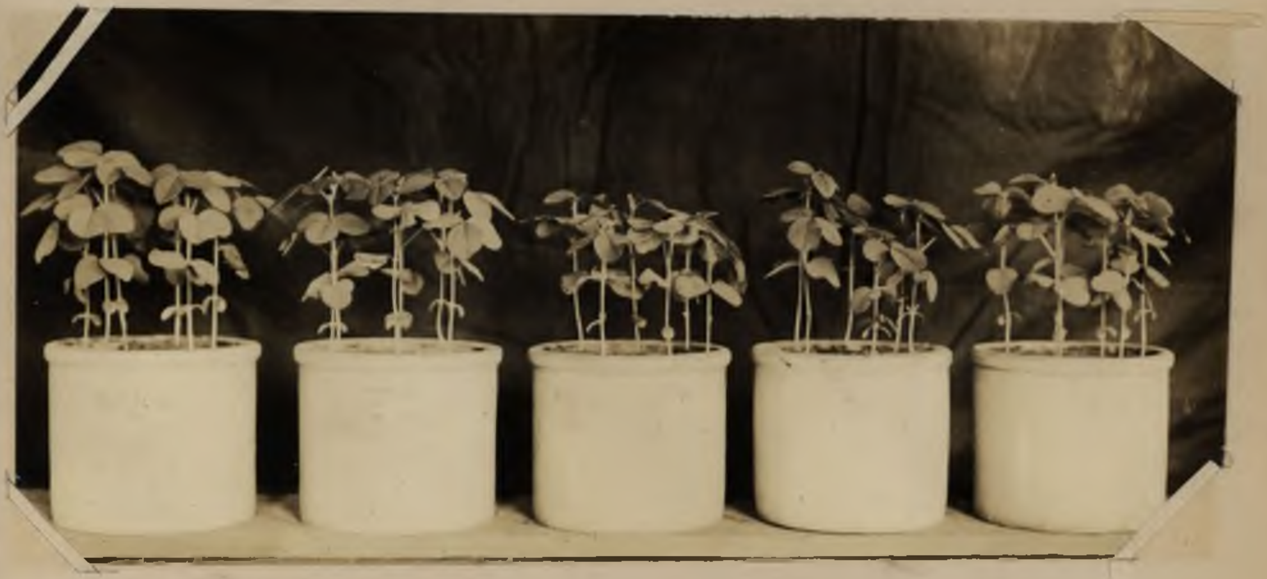


Fig.2. Plants to which sulfur alone was added, just before harvesting.

The condenced figures of the combined dry weights, pH values at begin and at the end of the experiment, avarage height,number of pods produced and total transpiration (plus evaporation) are given in table 6.

Yields of tops of second crop of soybeans grown in orange grove soil for 6 weeks

No.	Dry weight in grams	Relative weight none = 100	Transpiration in cc.	Average height in c.m.	Number of pods	pH begin	pH end	Remarks.
1	3.0302	100	4150	26.5	10	6.9	6.6	
2	2.9453	97.3	4170	26.5	15	6.9	6.5	
3	2.1595	71.4	3710	21	2	6.6	6.3	
4	2.2741	75.1	3535	21	6	6.6	6.1	Slightly injured
5	2.0000	66.0	3305	21.5	5	6.2	6.0	Strongly injured cotoledous green
In addition 300 pounds of rock phosphate								
6	3.2750	100	3875	21.5	13	7.0	6.7	
7	2.9348	89.6	3710	22	12	6.9	6.4	
8	2.8441	86.9	3755	23	10	6.8	6.2	
9	2.1642	66.2	3390	22	7	6.5	6.1	
10	1.3470	41.1	3080	19	0	6.1	4.6	Strongly injured cotoledous green
In addition 100 pounds of acid phosphate.								
11	3.0227	100	3695	24	8	7.0	6.7	
12	3.2712	108.1	4030	26.5	15	6.9	6.5	
13	2.5400	84.0	3480	23	7	6.8	6.3	
14	2.4882	82.5	3505	23	8	6.6	6.1	
15	1.9160	63.3	3235	21.5	3	6.2	5.8	Injured green cotoledous.

The results obtained are much more pronounced than in the first series of experiments. The fact that inoculated sulfur and rock phosphate together produced poorer results than sulfur alone can not fully be explained by supposing that this particular soil does not respond to phosphorus, since the results obtained with the acid phosphate treated pot cultures indicate that some benefit might have been derived from the phosphorus treatment. The cause for the poorer results seems to lie in the greater acidity produced,

The root systems of the plants receiving 100 pounds of sulfur were, in general, more extensive tham those of the plants in the check pots, but the roots of the plants in pots with higher sulfur applications were considerable less extensive. Inoculated sulfur applied in moderate quantities, therefore, might aid the plants to get hold of more plant food through stimulation of root development.

The number of nodules increased with the application of 100 pounds of sulfur but decreased considerably if greater quantities of sulfur were employed. The size of these nodules increased with the decrease of their numbers. The relative number of nodules for the different cultures, placing the checks at 100, were as follows:

Culture number	Nodules	Culture number	Nodules	Culture number	Nodules
1	100	6	100	11	100
2	120	7	150	12	120
3	100	8	100	13	120
4	80	9	80	14	100
5	20	10	10	15	60

The decrease in numbers of nodules for the plants receiving acid phosphatewere not as great as for the plants without phatsphate, and this can be attributed to the available phosphorus since the plant receiving rock phosphate decreased in numbers in the same manner as without phosphorus, and although the catual acidity of culture number 5 was even much less than of culture number 15, more nodules were produced in the latter. The numerical decrease of the nodules is undoubtedly due to the sulfate formation and the consequent increase of acidity.

B. VEGETATION EXPERIMENTS WITH SOIL No.2.

Similar experiments were made with soil number 2, which was poirer in nitrogen and mineral food constituents. Soy beans were planted in the same manner as has been preveously been described, and sulfur, rock phosphate and acid phosphate employed at the same rates.

The changes in acidity were in this case quite similar to series I as can be seen from table 7.

SERIES II TABLE 7

Hydrogen-ion concentrations, expressed in pH values, of soil water extracts before and during the growth of soybean plants.

		After weeks ;									
No.	Sulfur added in pounds per acre	0	1	2	3	4	5	6	7	8	9
1	None	7.0	6.6	6.6	6.6	6.8	6.8	6.9	6.8	6.8	6.7
2	100	6.9	6.6	6.6	6.5	6.7	6.7	6.7	6.8	6.7	6.7
3	300	6.8	6.4	6.4	6.4	6.5	6.7	6.8	6.7	6.6	6.5
4	500	6.9	6.5	6.5	6.5	6.5	6.5	6.4	6.3	6.2	6.0
5	1000	6.9	6.3	6.2	6.3	6.3	6.1	6.1	6.0	5.7	5.3
			In addition to sulfur 300 pounds rock phosphate								
6	None	7.0	6.8	6.7	6.6	6.5	6.7	6.7	6.7	6.7	6.6
7	100	6.8	6.6	6.6	6.5	6.7	6.6	6.5	6.8	6.6	6.4
8	300	6.8	6.6	6.5	6.5	6.5	6.4	6.5	6.4	6.4	6.3
9	500	6.9	6.6	6.5	6.4	6.4	6.3	6.3	6.3	6.3	6.3
10	1000	7.0	6.6	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.1

SERIES II TABLE 7 CONTINUED

		After weeks.									
No.	Sulfur added in pounds per acre	0	1	2	3	4	5	6	7	8	9
		In addition to sulfur 100 pounds of acid phosphate.									
11	None	6.9	6.7	6.6	6.7	6.8	6.7	6.7	6.7	6.7	6.7
12	100	7.0	6.6	6.6	6.6	6.7	6.7	6.6	6.7	6.7	6.6
13	300	6.9	6.7	6.7	6.6	6.5	6.5	6.4	6.4	6.4	6.3
14	500	6.9	6.5	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.3
15	1000	6.9	6.7	6.4	6.4	6.3	6.1	6.1	6.0	5.9	5.8

Here, however, the changes were greatest in the cultures to which sulfur alone was added, After 3 weeks the plants were, in general, much poorer than the plants grown in series one. The leaves of nearly all plants had yellow spots, and some of them wre more or less drooping. The general appearance and avarage height wre considerable inferior after 6 weeks when 3 of the 6 plants from every culture were harvested, then was the case with plants grown in soil number 1. The effect of sulfur was more pronounced and cultures receiving 1000 pounds of sulfur per acre were severely injured. The condenced data secured after 9 weeks when all plants were harvested are given in table 8.

TABLE 8

Yields of tops of soybeans grown in soil, which had received no fertilizer for more than 30 years.

No.	Dry weight in grams	Relative weight none = 100	Total Transpiration in cc.	Average height in c.m.	Number of pods	pH begin.	pH end.	Remarks
1	3.2745	100	3535	28.4	7	7.0	6.7	
2	3.4375	106.6	3498	29.2	5	6.9	6.7	dark green
3	3.1763	97.6	3555	26.4	5	6.8	6.5	
4	3.1026	95.4	3204	24.0	4	6.9	6.0	
5	2.9650	91.2	3041	26.4	4	6.9	5.3	poorest plants
			In addition 300 pounds of rock phosphate					
6	3.4122	100.0	3445	22.4	0	6.9	6.6	Blooming
7	3.4654	101.4	3531	24.0	2	6.8	6.4	
8	3.0996	91.0	3053	24.0	3	6.8	6.3	
9	3.3965	99.4	3236	27.2	3	6.9	6.3	
10	3.2862	95.4	3214	24.8	3	7.0	6.1	yellow leaves.
			In addition 100 pounds of acid phosphate					
11	3.6887	100.0	3979	29.6	9	6.9	6.7	
12	3.6965	100.3	3482	28.0	2	7.0	6.6	
13	3.3012	89.4	3314	28.0	5	7.0	6.3	
14	3.2381	87.5	3447	26.0	12	6.9	6.3	
15	3.3400	90.6	3242	24.0	3	6.9	5.8	earliest mature.

The soil responded far better to the treatment of phosphorus tham soil number 1. The cultures receiving acid phosphate and rock phosphate produced in general higher yields than the cultures to which sulfur alone was added. In all cases again sulfur applications of 100 pounds to the acre gave slightly increased yields.

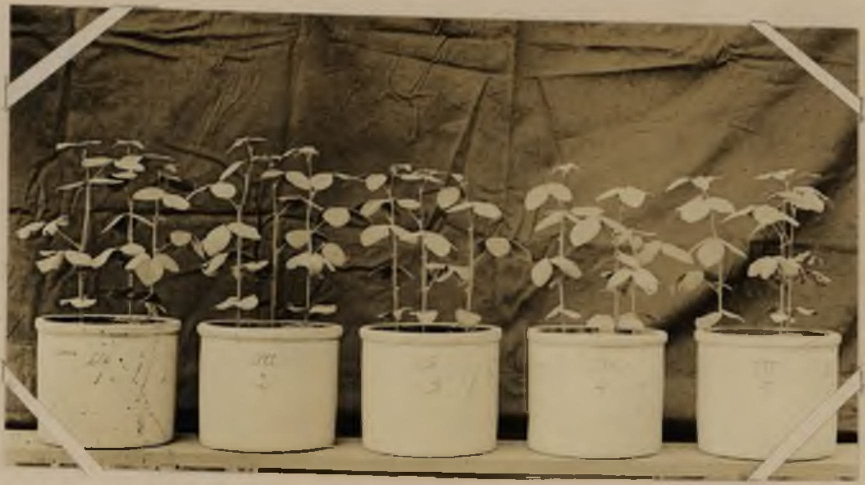


Fig.3. Soy bean plants,to which sulfur alone was applied,just before harvesting.

All plants were scored for general appearance, yellowness and stockiness before harvesting. The scores show even more pronounced than the dry weights of the plants that all cultures receiving 100 pounds of sulfur were better looking, greener and less spindling plants than the check plants or the plants grown in pots which received higher applications of sulfur.

A comparison of the nodules and root system brought out the facts as were found in soil number 1, but in a higher degree. The extent of the root system and also the number of nodules produced decraesed with the increase of the sulfur applications except the nodules on plants in cultures 2,7 and 11, which were relatively greater.

Since the data secured did not seem conclusive a second crop of soy beans was planted and applications of sulfur, rock phosphate and acid phosphate made as has been preveously mentioned in series I.

Changes in hydrogen-ion concentartion during the growth period are tabulated and given in table 9.

SERIES II a TABLE 9

Hydrogen-ion concentrations, expressed in pH values, of soil water extracts before and during the growth of the second crop of soybean plants.

No.	Sulfur added in pounds per acre	After weeks .						
		0	1	2	3	4	5	6
1	None	6.7	6.7	6.7	6.8	6.8	6.8	6.5
2	100	6.7	6.6	6.6	6.6	6.6	6.5	6.2
3	300	6.5	6.4	6.5	6.5	6.2	4.8	5.0
4	500	6.0	6.0	6.2	5.5	4.6	4.6	4.9
5	1000	5.3	5.2	4.6	4.7	4.2	3.9	4.0
		In addition to sulfur 300 pounds rock phosphate						
6	None	6.9	6.6	6.6	6.6	6.5	6.8	6.3
7	100	6.4	6.4	6.6	6.5	6.3	6.1	6.0
8	300	6.3	6.4	6.5	6.5	6.2	6.1	6.0
9	500	6.3	6.3	6.0	6.2	6.2	5.9	5.5
10	1000	6.1	5.5	4.6	4.5	4.6	3.8	3.8
		In addition to sulfur 100 pounds acid phosphate						
11	None	6.7	6.6	6.7	6.8	6.6	6.8	6.4
12	100	6.6	6.5	6.6	6.5	6.4	6.2	6.1

SERIES II a TABLE 9 CONTINUED

No.	Sulfur added in pounds per acre	After weeks							
		0	1	2	3	4	5	6	
13	300	6.6	6.5	6.6	6.5	6.2	6.1	6.0	(In addition to sulfur 100 pounds acid phosphate .)
14	500	6.3	6.3	6.3	6.5	6.2	6.0	5.7	
15	1000	6.3	6.2	5.8	5.8	4.4	4.4	4.5	

The hydrogen-ion concentration in this series was considerable higher (lower pH values)as was the case for the second crop in series I. Here again,the highest concentration found was in number 10,which received besides 1000 pounds of sulfur 300 pounds of rock phosphate. A number of the cultures were apparently less acid after after 6 weeks than at the end of 5 weeks.

Notes taken a few days before harvesting show that the cotolydons of the cultures receiving 300 and 500 pounds of sulfur had in nearly all cases been dried out or dropped, while cultures receiving higher applications of sulfur had still very dark green cotolydons. Culture numbers 3,8 and 13 seemed most mature,while cultures numbers 5,10 and 15 were severely injured. Dry weights of plants and other data secured are given in table 10.

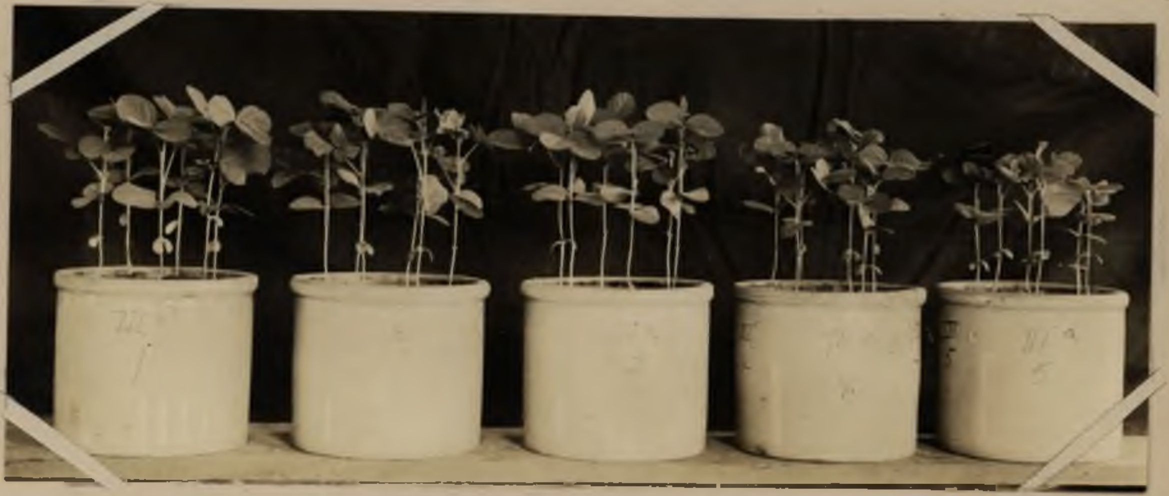


Fig.4.Soy beans with sulfur alone as they appeared just before harvesting.

TABLE 10

Yields of tops of second crop of soybeans grown in soil receiving no fertilizers for more than 30 years.

No.	Dry weight in growth	Relative weight none= 100	Total Trans-piration in cc	Average height in c.m.	Number of pods	pH begin.	pH end	Remarks
1	2.5990	100	3800	21.5	5	6.7	6.5	
2	2.4328	93.6	3500	21.6	5	6.7	6.2	
3	2.2447	86.5	3245	22.0	7	6.5	5.0	
4	1.9295	74.3	3110	19.0	5	6.0	4.9	
5	1.1808	45.4	2595	16.5	1	5.3	4.0	2 plants blooming, strongly injured.
In addition 300 pounds of rock phosphate								
6	2.3855	100.	3665	22.0	9	6.9	6.3	
7	2.3700	99.5	3345	23.4	8	6.4	6.0	
8	2.1425	90.1	3435	21.5	4	6.3	6.0	
9	1.9261	80.8	2880	19.0	7	6.3	5.5	
10	1.1520	48.4	2715	13.5	2	6.1	3.8	strongly injured.
In addition to sulfur, 100 pounds of acid phosphate								
11	2.2160	100.	3395	21.5	10	6.7	6.4	
12	2.5500	114.6	3445	24.2	10	6.6	6.1	
13	2.1645	97.4	3330	22.4	8	6.6	6.0	
14	1.9410	87.3	3545	19.0	7	6.3	5.7	
15	1.9863	89.2	3320	18.5	5	6.3	4.5	medium injured.

It is apparent that plants grown in soil receiving acid phosphate in addition to sulfur were best. The whole series which received rock phosphate in addition to sulfur were poorer than the cultures receiving sulfur alone. The acidity produced in these pots did not seem to enough to make the roch phosphate available, although some of the injury to the plants can be attributed to the acidity. Still this does not explain the phenomona in full. It very likely therefore, that the relatively high amounts of sulfates formed were harmfull or prevented the plants from taking up the necessary food constituents. The results with this soil poor in nitrogen and mineral food constituents brings out clearly the fact that the acidity produced was not able to supply greater quantities of the necessary plant food elements.

The nodule formation was also influenced by the addition of sulfur as can be seen by a relative comparison. If the check plants are placed at 100 the following figures were secured:

Culture number	Nodules	Culture number	Nodules	Culture number	Nodules
1	100	6	100	11	100
2	100	7	150	12	100
3	60	8	110	13	90
4	30	9	80	14	70
5	5	10	0	15	60

Sulfur added in large quantities depressed the formation of nodules considerable. The phosphate together with sulfur seemed either to have no influence, or were rather depressing. This may be seen if the relative numbers of nodules produced on the roots of plants to which sulfur alone was given are compared with the nodule formation on roots of plants which received phosphate in addition. The figures were:

Culture number	Nodules	Culture number	Nodules	Culture number	Nodules
2	100	7	80	12	90
3	100	8	70	13	90
4	100	9	100	14	60
5	100	10	0	15	100

This seems to point towards a certain degree of stimulation by small additions of sulfur, but depression when the acidity produced becomes to great.

C. EXPERIMENTS IN SAND CULTURES.

The acidity produced by inoculated sulfur in the soil cultures seemed not sufficient to attack the rock phosphate added.

To test out whether or not a similar phenomonon of sulfate production from inoculated sulfur in the presence of rock phosphate could be intensified, whereby the phosphorus would become more rapidly available, and to determine the effect of this intensified process on plant growth, soy beans were grown in washed quartz sand, using Shive's cultural solution (R_5C_2) as a basis.

The phosphorus of the cultural solution was substituted by Florida soft rock phosphate. The rock phosphate and inoculated sulfur had been mixed about 6 weeks previous to the application, kept in tumblers in an incubator, and tested for accumulation of acidity and for formation of sulfates. From these tests it was apparent that sulfur oxidation had begun. The mixture was added at arate of 2 tons per acre, being approximately the calculated amount of phosphorus in the check (Shive's R_5C_2 solution) cultures.

Hydrogen-ion concentration determinations were made at the begin of the experiment and regularly at the end of each week during the period which the plants were growing.

The results obtained are given in table 11.

Table 11

Soy bean plants grown in sand cultures for 6 weeks **with** inoculated sulfur and rock phosphate, and Shive's cultural solution as a basis.

Culture number	Additions	Weigth in grams	Average length in cm.	Number of pods	pH begin	pH end
1	None	0.850	8	bloom	5.8	6.8
3	Rock phosphate	1.454	11.5	bloom	5.4	6.6
5	R5C2	3.610	15.0	4	5.3	6.6
6	R5C2, rock phosphate	2.223	15.0	2	5.0	6.4
8	R5C_2, inocul. sulfur, rock phosphate	0.600	dead	-	4.7	2.9
14	R5C_2, inocul. sulfur.	0.811	dead	-	5.2	4.3

The inoculated sulfur with the rockphosphate mixed previous to the application which was substituted for the phosphorus in the cultural solution, had, at the end of 6 weeks, produced a **fair** growth of these plants. In the cases where inoculated sulfur was added the high acidity had killed the plants after about 14 days. The soy bean plants grown in Shive's nutrient solution to which inoculated sulfur was added made a good growth at first, but in 3 weeks the plants, which till then had survived, died. The roots were dark brown, with the appearance of being burnt.

Sulfur oxidation went on rapidly in the cultures to which the inoculated sulfur-rock phosphate mixtures were added as is indicated by the lowering of the pH values. The inoculated sulfur alone added to Shive's nutrient solution had also a decided influence on the lowering of the pH values,but no such great differences occured as in the cultures to which previously mixed inoculated sulfur and rock phosphate was added. This could be expected since sulfur oxidation had begun in the mixtures. Nevertheless, the plants grown in all these cultures were killed in a comparitively short time.

In the cases were no sulfur was added the plants made the solution int he sand less acid,bringing it nearly to the neutral point. The changes in H-ion concentration were regular as is shown by the pH determinations,which were made at definite intervals.

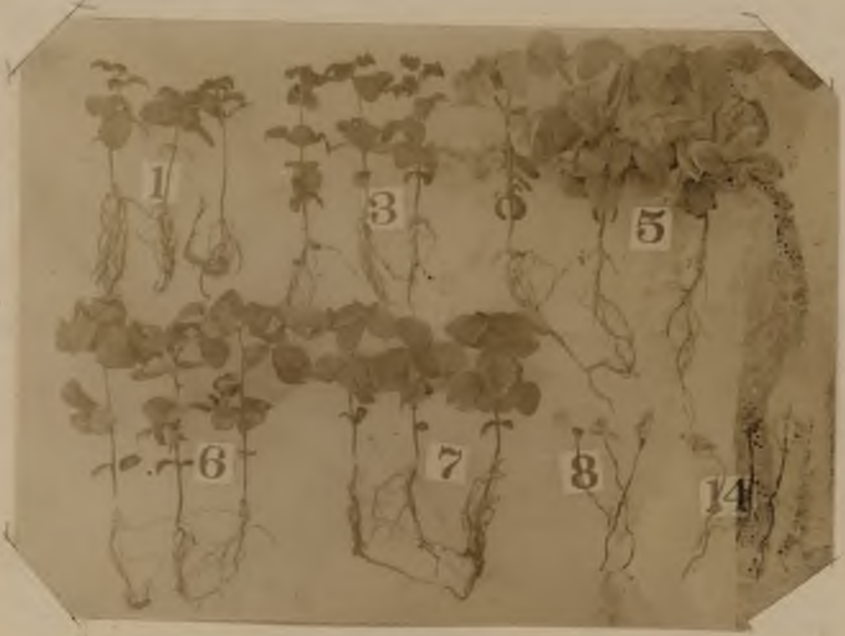


Fig.5.Soy bean plants grown in sand cultures with inoculated and rock phosphate.

Apparently not sufficient acidity was produced in the soil cultures earlier reported, since the acidity at which the phosphorus of the rock phosphate becomes soluble had not yet been reached. In the case of the sand cultures this point was reached, but the plants were not able to survive. The fact that very little or no phosphorus is shanged to a soluble form, has been found in the studies reported in part I. To find the exact acidity necessary to change Florida soft rock phosphate into soluble P_2O_5, a curve was constructed from readings of pH values obtained by adding different amounts of 0.1normal and normal sulfuric acid to the rock phosphate. The rock phosphate used for the construction of the curve was of the same lot which was added to all pot cultures.

Ten grams of rock phosphate were shaken with definite amounts of sulfuric acid for 2 hours in a shaking machine and left standing over night. An aliquot of the supernatent liquid was then drawn of and hydrogen-ion determinations made. At the critical point sufficient determinations were made to check up all points obtained. The results are graphically shown in fig.6.

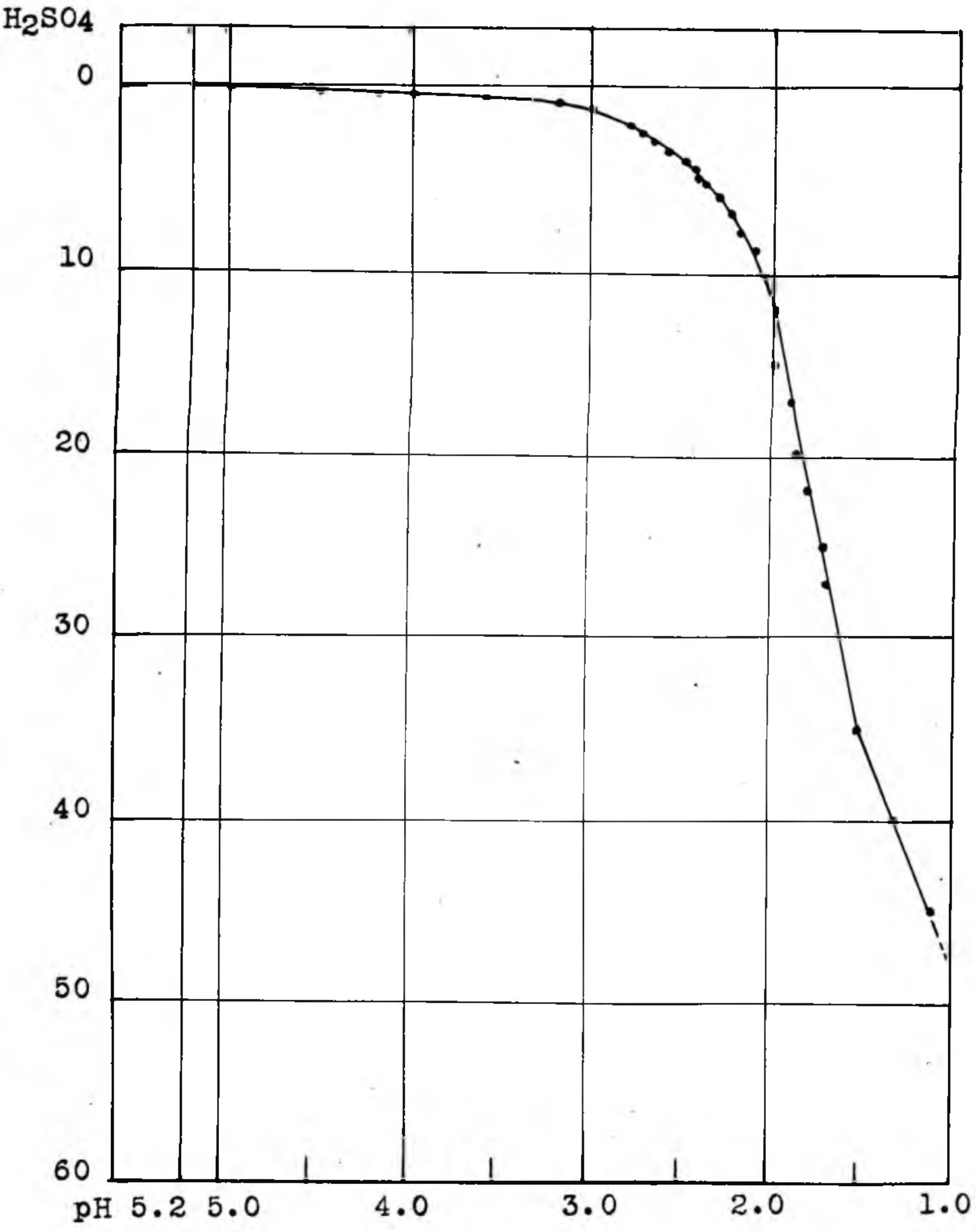


Fig.6. Graph showing the hydrogen-ion concentration at which rock phosphate becomes available.

The curve shows that the phpsphorus of the rock phosphate becomes rapidly available when a point is reached between pH 3.1 to 2.8.

Since under ordinary circumstances not sufficient acid is produced in the soil it is very doubtful whether greater beneficial effects can be expected from a mixture of rock phosphate and sulfur, which have not been composted, previous to the application, long enough so that most of the sulfur is transformed into sulfates and which has reacted with the phosphorus of the rock phosphate. It is very likely that the free acid produced by the sulfur oxidation is harmful to plant growth, more than the total amounts of sulfates or the so called total acidity. Beneficial effects might de derived from inoculated sulfur which has not been composted with rock phosphate but mixed with the rock phosphate before applications are made, and when applied in small quantities, if the soil is in need of sulfur so that plant growth is stimulated. The plants under observation seemed to be stimulated by the sulfur to make more extensive root systems and thus would be able to take up greater amounts of plant food from the soil solution or from the slowly available rock-phosphate present. At the same time the acidity produced would be not too intensive to be harmful to the normal development of roots and tops. It seems ,however,more safe to compost the sulfur-rock phosphate some time before application untill most of the sulfur is converted into sulfates and has reacted with the phosphorus of the rock phosphate,in order to avoid the injurious effects.

SERIES II. BIOLOGICAL EXPERIMENTS.

A. Soil number 1.

In several instances it has been reported that sulfur and sulfates exerted a stimulating influence on bacterial growth. The possible inadequate amounts of sulfur in soils is apt tp produce an influence upon micro-organisms,and following the addition of certain quantities of it,a change in the biological flora can be expected.

The work reported in this part of the paper was mainly carried out to study the change in bacterial numbers and but slight attentiom was paid to the occurance and determination of different species.

The amounts of sulfur added to the soil are given in the tables. During the incubation period hydrogen-ion concentration determinations of the soil water extract were made at frequent intervals. The data secured is given in table 12.

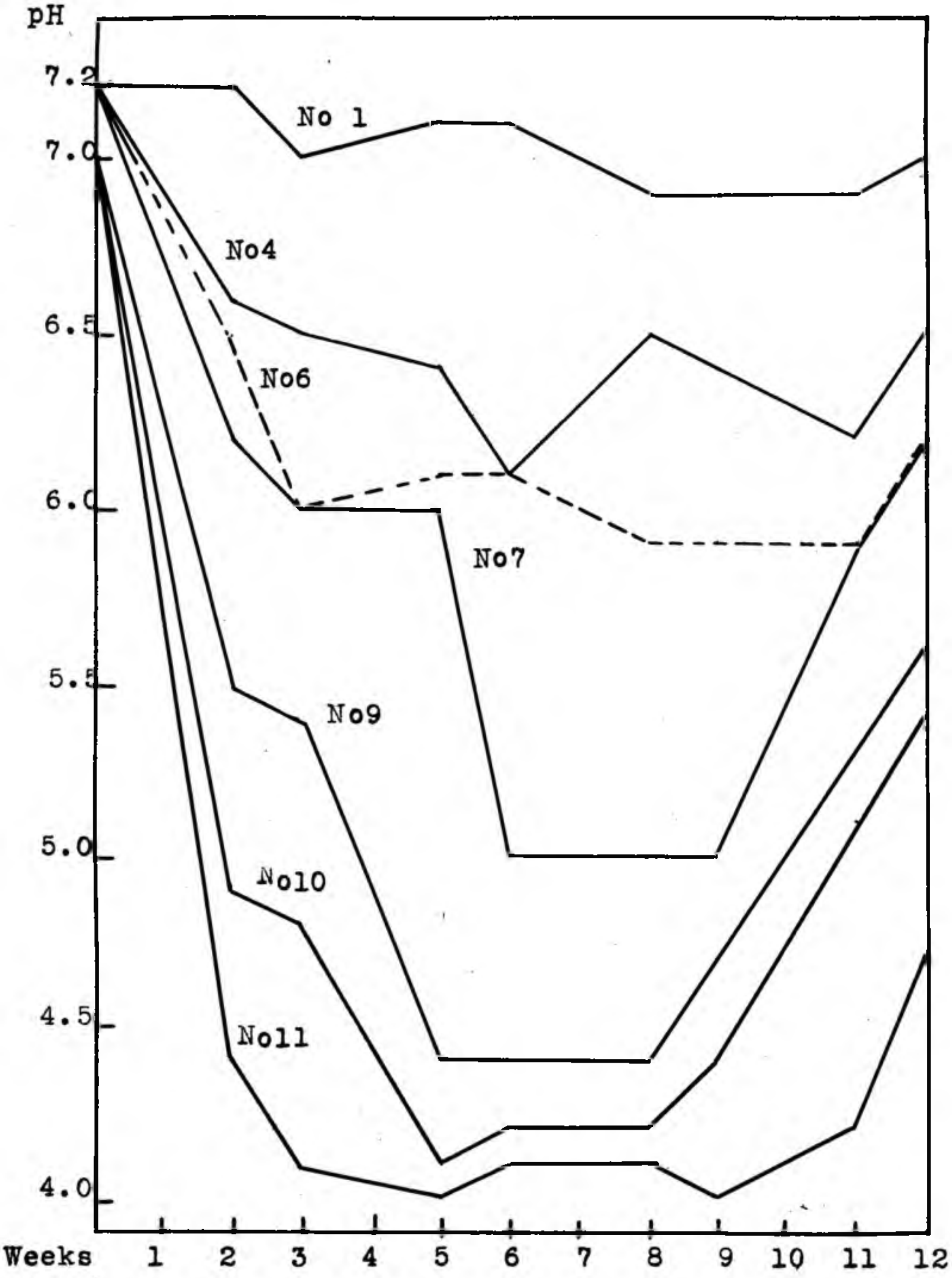
TABLE 12

Changes of hydrogen ion concentrations of water extracts from Soil No. 1 during the period of incubation.
(Expressed in pH values)

Culture number	Pounds of sulfur per acre	After weeks								
		0	2	3	5	6	8	9	11	12
1	none	7.2	7.2	7.0	7.1	7.1	6.9	6.9	6.9	7.0
2	100	7.2	7.3	7.2	7.2	6.9	7.1	6.7	6.9	6.9
3	300	7.2	7.3	7.0	6.9	6.2	6.8	6.7	6.2	6.5
4	500	7.2	6.6	6.5	6.4	6.1	6.5	6.4	6.2	6.5
5	1000	7.2	6.7	6.7	6.3	6.2	6.5	6.3	6.4	6.5
6	1500	7.2	6.5	6.0	6.1	6.1	5.9	5.9	5.9	6.2
7	2000	7.1	6.2	6.0	6.0	5.0	5.0	5.0	5.9	6.2
8	2200	7.0	5.9	5.9	5.7	4.5	4.8	4.8	5.4	5.8
9	2500	7.0	5.5	5.4	4.4	4.4	4.4	4.7	5.3	5.6
10	3000	7.0	4.9	4.8	4.1	4.2	4.2	4.4	5.1	5.4
11	3500	7.1	4.4	4.1	4.0	4.1	4.1	4.0	4.3	4.7

From the data presented it can be seen that in all cases the hydrogen-ion comcentration became graeter till a certain point was reaced, from then on the movement was back towards the neutral point. This turning point was reached in all cases before or between the 9th and the 11th week. of incubation. It should be remembered that no plants were growing in these tumblers. It seems therefore, that the sulfates formed reacted with the soil constituents bringing the soil solution back to a cerain equilibrium. This phenomonon is even more clearly demeonstrated by the curves of fig.7.

Fig.7. Curves showing changes of Hydrogen-ion concentrations of soil number 1, treated with different amounts of inoculated sulfur.



Bacterial numbers were determined by the plate method,using Lipman and Brown's synthetic agar,at the beginning of the experiment,after 6 weeks,and after 12 weeks.

The numbers in thousands per gram of soil are given in table 13.

TABLE 13

Bacterial numbers in soil number 1 treated with inoculated sulfur
(In thousands per gram)

Pounds of sulfur per acre	None	100	300	500	1000	1500	2000	2250	2500	3000	3500
Culture Number	1	2	3	4	5	6	7	8	9	10	11
Begin	3.280	3.240	3.190	3.200	3.195	3.260	3.240	3.275	3.260	3.235	3.250
6 weeks	3.195	2.520	3.470	4.470	4.160	3.600	2.675	2.390	1.935	1.405	1.390
12 weeks	3.200	3.525	3.615	3.325	3.165	2.720	1.840	1.415	1.345	765	560
18 weeks	3.170						2.950	1.950	550	450	355

The bacterial numbers in the untreated soil remained about constant during the incubation period. The biological flora in the soils treated with amounts of sulfur up to 1500 pounds per acre seemed to be stimulated for the first 6 weeks,but the numbers in soils treated with greater quantities showed a rapid decline. The higher the applications of sulfur and consequently the higher the amounts of acidity produced,the more sulfates there were formed,the fewer colonies were coumted on the agar plates. After 12 weeks much of the stimulating effect seemed to be lost in the cultures which had previously produced increased numbers. Applications of 100 and 300 pounds of sulfur to the acre seemed somewhat consistent in the production of higher numbers,but the decrease was very marked in the cultures receiving from 2000 pounds upward. The numbers in the cultures to which 3500 pounds of sulfur per acre was added had at the end of 12 weeks but slightly more than one sixth of the origional numbers. There is no doubt,however,that the depression of the origional biological flora was accompanied by an extraordinary increase in sulfur oxidizing organisms, but since these organisms do not reproduce on ordinary agar plated,or at least can not be counted in the ordinary way on account of their extremely small size,no estimation of their numbers could be made.

After 18 weeks of incubation the amounts of sulfur oxidized were for all cultures,which received the higher applications,greater,but the movement of the H-ion concentrations was still father towards the neutral point. The numbers of colonies counted on agar plates increased in two cultures,while others showed a greater decrease. Cultures to which 3500 pounds of sulfur were added had at the end of 18 weeks but slightly more than one tenth of the original numbers present.

B. Soil numbers 16 and 17.

In 1916 Lipman (16) made the suggestion that the change of sulfur to sulfuric acid in the soil may be employed for converting sodium carbonate into sodium sulfate and making productive the barren alkali areas. Lipman and Sharp (15) showed in their studies of chemical treatment of alkali land that where about 50 tons of strong sulfuric acid were sprayed on 40 acres of alkali soil there was a decided improvement in the productive power of the soil,but not great enough to justify the sulfuric acid treatment in practice. Treatment with sulfur gave no results.

For the study of the behavior of inoculated sulfur in black alkali soils,two 200 pounds samples of such soil were secured by courticy of Professor Hoagland of the University of California. For information concerning one of the alkali soils the following data was given by Proffessor Haogland:

"This soil was obtained from the University Ranch near Fresno in the San Joaquin Valley,California. It came from a bad spot in a tract of 160 acres called the "experimental drainage tract". This land was originally productive but later ceased to produce crops on account of the accumulation of the so-called alkali salts. Some years ago an attempt was made to reclaim this soil by installing a tile drainage system five or six feet deep and by flooding twice with an excess of good irrigation water. An attempt was then made to grow a barley crop but it was found that the reclamation was not successful as less than one half of the land was capable of even f fair production. Many spots were entirely bare."---"Our present information leads us to believe that it is almost impossible to remove the toxic alkalinity from this soil by ordinaty washing. It ~~is~~ undoubtedly contains various alkaline substances including sodium carbonate, sodium silicate, sodium organic compounds.etc. The physical state of the soil is exceedingly bad."

The given information is undoubtedly about soil number 17 for, although this soil will not support any ordinary growth as has been repeatedly tried with different crops in the greenhouse, and although it has a high alkalinity and a high content of alkali salts, the alkalinity and the amounts of salts are not as high as in soil number 16. Moreover, soil number 17 has a decidedly lower chlorine content than soil number 16. Both the soils are sandy loam soils with a very fine structure. This can be seen from the data given in table 14, showing the results of the mechanical analyses of these soils.

Table 14
Mechanical analyses of soils number 16 and 17

Soil	No16	No.17
Fine gravel	per cent 4.94	per cent 5.21
Coarse sand	11.14	12.07
Fine sand	7.85	9.56
Very fine sand	28.10	31.52
Silt and clay	47.56	41.42

The poor structure of this soil as well as the high alkalinity prevents ordinary crops from taking hold, and in addition to changing the alkalinity the structure simaltaniously mustt also be improved. No complete analyses of these soils were made but only as far as seemed necessary for the scope of the study under discussion.

The data involved are given in table 15.

Table 15

Analyses of soils numbers 16 and 17.

Soil	No.16	No.17
	per cent	per cent
CaO, MgO, Fe_2O_3	0.372	0.374
K_2O	1.12	1.16
P_2O_5	0.2656	0.2337
Cl	0.7384	0.0923
N	0.0581	0.0454
SO_4	0.097	0.076
Total alkalinity (0.1 norm. H_2SO_4)	46.81	35.20
pH	9.4	9.0

To the soils were added amounts of sulfur equal to those applied to soil number 1, and the highst amounts of sulfur added were calculated to be adequate for the neutralization of the high alkalinity of soil number 16. The different applications were made on purpose to see whether or not sulfur oxidation would occur in smaller quantities in these soils evidently toxic to the biological flora and to plant growth. Although the same quantities of sulfur were applied to soil number 17, the greater amounts were more than necessary to neutralize the alkalinity of the soil.

During the incubation period a record was kept of the changes in hydrogen-ion concentrations, which for soil number 16 are recorded intable 16.

It can be seen at once that the smallest sulfur applications excerted little or no influence on the change of the hydrogen-ion concentration, but the higher amounts of inoculated sulfur brought about a decided lowering of the hydrogen-ion concentration. However, after an incubation period of 12 weeks, the neutral point was be no means reached.

Much more pronounced were the changes of the H-ion concentration caused by sulfur oxidation in soil number 17, which changes are reported in table 17.

TABLE 16

Changes in hydrogenion concentrations of water extracts from soil No. 16 during the period of incubation.

(Expressed in pH values)

Culture number	Pounds of sulfur per acre	After weeks								
		0	2	3	5	6	8	9	11	12
12	none	9.8	9.7	9.7	9.8	9.9	9.9	9.9	9.7	9.6
13	100	9.8	9.7	9.6	9.5	9.8	9.8	9.8	9.6	9.7
14	300	9.8	9.4	9.5	9.5	9.7	9.8	9.8	9.6	9.6
15	500	9.8	9.4	9.6	9.6	9.8	9.8	9.7	9.7	9.6
16	1000	9.8	9.5	9.5	9.6	9.8	9.7	9.8	9.7	9.3
17	1500	9.8	9.8	9.7	9.6	9.6	9.6	9.6	9.5	9.3
18	2000	9.8	9.7	9.5	9.4	9.3	9.3	9.3	9.4	9.2
19	2250	9.8	9.7	9.4	9.2	9.3	9.3	9.3	9.4	9.1
20	2500	9.8	9.8	9.4	9.3	9.3	9.3	9.2	9.1	9.0
21	3000	9.8	9.6	9.4	9.3	9.2	9.2	9.3	9.1	8.9
22	3500	9.8	9.8	9.8	9.8	9.7	9.5	9.3	8.7	8.2

TABLE 17

Changes in Hydrogen-ion concentrations of water extract from soil No. 17 during the period of incubation.

(Expressed in pH values)

Culture number	Pounds of sulfur per acre	After weeks								
		0	2	3	5	6	8	9	11	12
23	none	9.0	9.1	9.0	9.0	9.0	9.0	9.0	9.0	9.0
24	100	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.9	8.8
25	300	9.0	9.0	9.1	9.0	9.0	9.0	9.0	8.8	8.6
26	500	9.0	9.0	8.7	8.9	9.0	8.9	9.0	8.6	8.4
27	1000	9.0	9.0	8.6	8.3	8.6	8.5	8.5	8.2	8.0
28	1500	9.0	8.8	8.1	7.8	7.7	7.4	7.4	7.4	7.4
29	2000	9.0	7.7	7.8	8.0	8.1	8.1	8.0	7.9	7.4
30	2250	9.0	7.6	7.5	7.4	7.3	7.3	7.3	7.2	7.0
31	2500	8.9	7.5	7.4	7.6	7.5	7.4	7.3	7.3	7.0
32	3000	8.9	7.4	7.4	7.4	7.3	7.2	7.1	7.0	6.8
33	3500	9.0	7.5	7.4	7.3	7.2	7.0	6.9	6.3	6.6

The changes in H-ion concentrations occuring during the relatively short incubation period of 12 weeks were such, that in several cultures the neutral point was reached before or at the end of this period. This indicated that if sufficient inoculated sulfur is added the suggestion made by Lipman, that the sulfur changing to sulfuric acid could be employed to convert the sodium carbonate into sodium bi-carbomate and sodium sulfate, might possibly successfully be brought into practice. The results would be noticed within a reasonably short time, provided the soil had been sujected to leaching before the sulfur treatment.

These experiments were repeated which a second lot of two samples of about 100 pounds each, sent by courticy of Dr. Hoagland from the same place. The soils were again labelled 16 and 17. For conveniency they will be refferred to as numbers 16a and 17a. These experiments were conducted in the greenhouse, using glaced earthenware pots with a capacity of 2.5 kilograms. To the soils were added the following quantities of sulfur:

CultureNo.	Pounds per acre	Culture number	Pounds per acre	Culture number	Pounds per acre
1,10	none	4,13	1000	7,16	2500
2,11	300	5,14	1500	8,17	3000
3,12	500	6,15	2000	9,18	3500

The pots were left standing on a bench in the greenhouse and watered at definite intervals. Soon heavy salt incrustations were formed at the surface, but the soil was not stirred and no attempt was made to remove the crystalized salts other than by adding water. This may explain why slight irrigularities occured in the readings of the hydrogen-ion concentrations. The changes recorded are reported in table 18.

TABLE 18

Changes in Hydrogen -ion concentrations of Soils number 16a and 17 a treated with inoculated sulfur and placed in the greenhouse.

Soil	Culture Number	Pounds of Sulfur per Acre	After Weeks									
			0	1	3	5	6	7	8	10	11	12
16 a	1	None	9.2	9.2	9.1	9.2		9.2		9.2	9.2	9.1
	2	300	9.2	9.1	9.2	9.1		9.0		9.0	9.1	9.2
	3	500	9.2	9.1	8.9	9.0		8.9		8.8	8.9	9.1
	4	1000	9.1	9.1	9.1	8.9		9.0		8.4	8.5	8.6
	5	1500	9.2	9.2	8.9	8.7		8.4		8.2	7.9	7.6
	6	2000	9.1	9.1	8.8	8.1		8.0		8.0	7.9	7.9
	7	2500	9.2	9.1	8.6	8.1		8.1		7.7	7.6	7.6
	8	3000	9.2	9.0	8.5	7.9		8.1		7.5	7.6	7.7
	9	3500	9.2	9.0	8.2	7.7		8.0		7.6	7.6	7.5
17 a	10	None	9.2	9.2	9.0	9.2	9.1	9.2	9.0		9.0	9.2
	11	300	9.2	9.2	9.2	9.1	9.0	8.8	8.6		9.0	9.2
	12	500	9.1	9.2	9.0	8.9	9.0	8.6	8.6		9.0	9.1
	13	1000	9.2	9.1	8.9	8.6	8.8	8.4	8.4		8.8	8.6
	14	1500	9.2	9.1	9.1	8.7	8.6	8.0	8.0		7.7	7.9
	15	2000	9.2	9.1	9.0	8.5	8.6	8.0	8.0		8.0	7.9
	16	2500	9.1	9.2	9.0	8.4	8.4	7.7	7.5		7.4	7.5
	17	3000	9.1	9.2	8.8	8.2	8.2	7.9	7.5		7.3	7.4
	18	3500	9.1	9.1	8.7	8.2	8.1	7.5	7.3		6.6	6.8

The changes of H-ion concentration in these soils placed in pots were very similar to the changes brought about by inoculated sulfur in the tumblers kept at a supposed optimum moisture content and a regulated temperature. Slight changes took place in the soils to which 300 and 500 pounds of sulfur to the acre were added, till the end of the 8th or the end of the 10th week, particularly in soil number 17a. After this time the H-ion concentration became less, and turned back towards the original concentration. As could be expected the higher application of sulfur excerted a far greater influence on the change of the H-ion concentration than the small amounts added. After 12 weeks the neutral point was reached in the case of soil number 17a to which 3500 pounds of sulfur was added, and where smaller quantities of sulfur were applied the H-ion concentration approached the neutral point.

These soils do not support any ordinary plant growth. An attempt was made to determine at what H-ion concentration barley seedlings would survive and start to grow. Every 14 days barley seedlings were planted in all pots, during the first 10 weeks without being able to keep the seedlings alive. In the soils to which large quantities of sulfur was added the plantlets made considearble growth but owing to the small capacity of the pots and the imperviousness of the soil the plants were injured by the salt solution concentrating at the surface.of the soil. The pots were watered frequently and the water added dissolved the hard alkali incrustations formed on top of the soil,and because of the poor physical condition of the soil this solution remained on the surface a considerable lenght of time and finally killed them.

Barley seeds were then planted,and they germinated well insoil number 17a receiving 1500 pounds or more of sulfur per acre. Only a few seeds germinated in soil number 16a to which 2500 or more pounds of sulfur per acre was applied. All these seedlings grew well,had a very dark green colour,but most of them were not able to survive the injury caused by the constant flooding and consequent attack of the concentrated salt solution. It seems reasonable to expect far better results in the field, or in pots with a device to take care of the watering or at least of the surplus of water. These soils have an extremely poor structure and would be difficult to handle even without injurious "alkali" salts.

For the study of the biological changes infusions were made from soils number 16 and 17, incubated at 28°C, and plated out on Lipman and Brown's agar at the beginning of the experiment, after 6 weeks and after 12 weeks. The biological flora for these soils expressed in numbers of colonies growing on agar plates varied directly as the H-ion concentration. The numbers of colonies per plate as an average of three plates counted is given in table 19.

TABLE 19
Bacterial numbers in soil numbers 16 and 17 treated with inoculated sulfur (in thousands per gram).

Soil No.	Pounds of Sulfur per acre	None	100	300	500	1000	1500	2000	2250	2500	3000	3500
	Culture Number	1	2	3	4	5	6	7	8	9	10	11
16	Begin	60	58	64	62	63	60	59	60	58	62	60
	6 weeks	61.5	30	35	65	50	60	181	231	230.5	226	255
	12 weeks	69	75	105.5	125.5	107	112.5	208	251	302	348	411
17	Begin	335	332	333	334	335	332	318	329	326	334	329
	6 weeks	330	325	320	275	260	250	225	255	250	235	255
	12 weeks	435	442	490	540	630	960	1.700	1.225	1.550	1.600	2.100

The increase in colonies was quite consistent with the increase of the amounts of sulfur added. Still the highest numbers obtained were relativily small if compared with the numbers present in ordinary soils. The numbers in the untreated incubated soils seemed to increase slightly as could be expected from the favorable temperature and moisture content. After an incubation period of 12 weeks,the soil receiving 3500 pounds of sulfur per acre,was found to produce approximately five times as many colonies per plate as the untreated soil. The numbers for the cultures with the higher sulfur applications for soil number 17 were from 3 to 5 times greater.

It was noted throughout all the experiments in the 3 different soils to which certain quantities of sulfur were added that a relativily large number of tiny white colonies of actinomyces appeared on the surface of the soil. In fact,if the soils were very acid or extremely alkaline,the counts made on the agar plates usually consisted for the most part of molds and actinomyces. The molds predominated in the cultures to which very small amounts of sulfur were added and the actinomyces in the cultures with greater quantities of sulfur. Often two-thirds or even four-fifths of the colonies on the plates inoculated with infusions from the soil with the lower sulfur applications, were molds and actinomyces. In the alkaline soil number 17 to which larger amounts of sulfur were added and which reached or approached the neutral point, but a very small number of the total colonies counted consisted of molds and actinomyces. It was surprising to notice the difference which occured. Nevertheless, not a great variety of biological flora was present.

SERIES III. INFLUENCE OF SULFUR ON THE PHYSICAL STRUCTURE OF THE SOILS.

The influence of chemicals, salts and various substances upon soils has been studied in innumerable instances. There is no doubt about the important part played by the various constituents added to a soil. The work reported in this part of the paper was carried out mainly to study the change in the physical stucture of the soils when inoculated sulfur was added, and transformed into sulfates. In the coarse of the biological and vegetation experiments it was frequently noticed that the soil, to which different amounts of sulfur were added, has a tendency to form small aggregates, resulting in making the soils apparently more porous.

Unfortunately no good methods are available for measuring accurately the changes in granulation. The methods employed to estimate small differences caused by additions of salt could not be used on account of obvious obstacles. An attempt was made to determine differences in granulation ,produced as a result of sulfur oxidation,by screening the soils,but slight differences in the moisture contents of the soils produced greater errors than the differences found between the soils treated with varying amounts of inoculated sulfur. Drying the soils in the oven made no difference in the size of the errors.

An effort made to increase the differences in cohesion of the soils as described by Cameron and Gallagher (6),and again by Davis (7),by means of an apparatus for measuring penetration,failed likewise to gave reliable results.

Patten and Waggeman (22) have shown that adsorption of soluble material exerts an appreciable influence upon the water holding power of finely divided soils. The hydroxides and carbonates seemed to lower the water holding capacity and other salts to raise it. An attempt was made to determine the influence of the formation of sulfates in the soils,upon the water holding capacity.

The endeavor the measure the formation of aggregates, by measuring the capllary rise of water, failed because the soils had to be packed closely thereby destroying the small aggregated formed.

The measured changes from carbonates into bicarbonates, flocculation and turbidity measurements can hardly bring out the phenomonon of aggregate formation. Determinations of apparent specific gravity of the treated and untreated soils are also apt to produce errors, even if the samples are previously dried, since slight differences in the initial percentage of water may cause irrigularities.

Nevertheless, all these interdependant factors together, can give some idea of the changes caused by the oxidation of elemental sulfur into sulfates by microorganisms.

METHODS.

Water holding capacity determinations of the treated and untreated soil were made according to the Hilgard (12) method after 12 weeks of incubation. An average of three determinations are given in the tables.

The method used to determine the change in apparent specific gravity of the soils was that of weighing full of soil a vessel whose volume was known. The dried soil was allowed to fall through a coarse wire screen until it overran the edges of the cup, and was then carefully leveled to the top of the cup with the edge of a spatula as reccommended by Davis (7). After weighing the cup and soil, the top of the soil was taken off and a representative sample of the interior of the mass placed in a weighing bottle and the moisture determined by heating for 15 hours at 105 to 108°C. Triplicate moisture determinations showed that but little difference occured between the previously dried samples, and an average of these three determinations was taken as a basis for calculations.

Determinations of apparent specific gravity on samples not previously dried showed greater differences but were discarded. At least two determinations were made of all samples.

Flocculation of the soil was determined by shaking two grams of soil with 50 cc. of distilled water using burettes as containers, anxd the clear or transparant liquid, after standing for 24 hours, measured as accurately as possible.

Turbidity measurements were made by mixing one part off soil and two parts of water, the soil suspensions poured into burettes and after 24 hours the upper 10 cc.

carefully pipetted off into weighing dishes,and the total residue estimated after evaparation and drying at 100°C as has been suggested by Hoagland and Martin (13).

Total alkalinity and bi-carbonates were determined by making water extractions of 20 grams air dried samples of each soil. The samples were shaken with 200 cc. of distilled water in a shaking machine for two hours. The flasks were left standing over night and filtered until the liquid was clear or transparant without sediment. An aliquot was titrated with fiftiest normal sulfuric acid using methyl red and phenolphtaleinbas indicators.

In the cases of the alkali soils a few cc. of concentrated hydrochloric acid were added to the remainder of the liquid to aid settlement of possible small quantities of colloidal matter.

Soluble sulfate formed was determined in aliquots of the liquid to which were added 2 cc. concentrated hydrochloric acid,by precipitating at the boiling point with barium chlorid.

EXPERIMENTAL RESULTS.

A. Soil number 1.

The formation of sulfate in soils treated with inoculated sulfur is influenced by the water content of the soil. Experiments were carried out to determine at which point of water saturation sulfur oxidation would reached the optimum for the soils used. It was found that oxidation increased with the increase of moisture until approximately 55 per cent of the amount necessary for complete saturation was reached. From then on oxidation decreased slowly to about 70 per cent of the water holding capacity. When larger quantities of water were added hydrogen sulfide began to develop, which could easily be detected by the smell and sometimes even by the black colour produced in the mixtures.

It was found in earlier experiments that sulfur oxidation proceeded more rapidly at a temperature of 30°C than at room temperature. The cultures under discussion were kept at 28°C throughout the experiments. This temperature, however, does not necessarily mean best for most rapid sulfur oxidation.

TABLE 20

Formation of sulfates, changes in total acidity, flocculation, turbidity, water holding capacity and apparent specific gravity in soil No. 1, after 12 weeks of incubation.

Culture Number	Pounds of sulfur per acre	Milligrams sulfate	Total acidity cc.0.1 N NaOH	Trans-parent liquid in cc.	Turbidity (mgr)	Water holding capacity per cent	Specific gravity
1	None	1.07#	0.02#	7.5	24.2#	31.9	126.83
2	100	2.39	0.05	8.0	23.9	32.7	126.02
3	300	5.38	0.20	8.0	22.9	31.3	126.22
4	500	7.15	0.28	11.5	18.4	31.2	124.32
5	1000	15.68	0.30	12.0	13.2	31.4	123.12
6	1500	17.92	0.32	12.2	6.2	33.1	118.17
7	2000	20.52	0.40	14.1	4.7	31.4	113.08
8	2250	19.08	0.40	16.3	2.0	27.5	113.08
9	2500	24.20	0.50	24.2	2.2	28.7	114.81
10	3000	24.86	0.60	32.4	1.9	29.0	116.08
11	3500	27.80	0.64	+ 50.0	1.8	28.6	118.27

Per 10 grams of soil

The data given in table 20 show that with the increase of the amount of inoculated sulfur an increase occured in water soluble sulfates. The increase obtained, however, was not proportional to the increase of sulfur added, as is reported in another table.

With the increase of sulfate accumulation the titrable acidity naturally increased.

The soil to which inoculated sulfur was added seems to assume a new set of physical properties, characterized by a more or less complete deflocculation. The increase of sulfate accumulation was nearly proportional to the decrease in deflocculation, which is very rapid when greater quantities of sulfur are employed. From the data presented it is evident that there exists a good correlation between the quantities of suspended material as measured by the transparant liquid colomn and the quantities of this material weighed after drying (turbidity), and the amounts of sulfur oxidized.

A number of investigators have set forth the influence of various hydroxides, carbonates, phosphates, sulfates, etc. upon the water holding power of soils. Although the results obtained are irrigular, there seems to be a general tendency to decrease the water holding capacity with the increase of sulfates formed.

The data obtained and presented in table 20 for the changes in apparent specific gravity seems to indicate that the formation of sulfates caused the soil to increase in volume up to a certain point. After this point was reached the volume decreased again. It should be born in mind, however, that not all the sulfur added had been oxidized, and the quantities of oxidized sulfur necessary to bring about greatest increase in volume may be different from the quantities applied where this point was observed and as is indicated by the figures in the table.

B. Soil number 16.

The oxidation of sulfur as indicated by the water soluble sulfates increased with the increase of the quantities of inoculated sulfur employed as shown in table 21.

It is very evident that the carbonates were transformed into bi-carbonates with the decrease in alkalinity. It is obvious that the toxic carbonates were changed to a great extent, in fact, almost all titrated alkalinity in the soil to which the largest amount of sulfur was added, had been converted into bi-carbonates,

TABLE 21.

Formation of sulfates and bicarbonates, changes in total alkalinity, flocculation, turbidity, water holding capacity and apparent specific gravity in soil No 16, after 12 weeks of incubation.

Culture Number	Pounds of sulfur per acre	Milligrams sulfate	Bi-carbonates	Total alkalinity cc 0.1 Norm. H_2SO_4	Transparent liquid in cc.	Turbidity (mgr) (Salts included)	Water holding capacity	Specific gravity
12	None	9.71[#]	1.04[#]	4.68[#]	1.0	231.2[#]	28.2	123.72
13	100	13.98	1.00	4.60	1.5	231.4	28.2	123.02
14	300	14.26	1.98	4.61	1.7	231.0	27.3	122.30
15	500	14.88	1.82	4.58	4.0	230.4	27.7	121.98
16	1000	15.72	1.72	4.52	4.0	230.0	26.6	121.55
17	1500	16.76	2.04	4.52	6.1	229.6	28.2	120.44
18	2000	26.32	2.54	4.47	5.2	229.0	28.9	118.88
19	2250	35.79	2.86	4.47	6.2	227.0	30.0	120.38
20	2500	37.38	2.79	4.32	7.1	227.1	28.0	115.96
21	3000	39.02	2.78	4.20	7.3	221.5	27.9	117.11
22	3500	42.38	3.20	3.60	9.2	221.0	30.7	115.38

[#] Per 10 grams of soil.

although the neutral point was by no means reached at the end of the incubation period of 12 wweks, and high alkalinity and large amounts salts still prevailed.

With the decrease of alkalinity an increase in flocculation occured, but this increase was, if compared with soil number 1, not very large. The figures showing turbidity measurements include the salts present after shaking with distilled water for two hours in a shaking machine, and therefore, do not represent the true turbidity, since the drying of the residue do not remove the salts present. It was thought better not to try to deduct the amounts of salts present by calculation, since the amounts of salts present would be necessarily different for the different culture numbers on account of the sulfur oxidized. Still, an appreciable decrease in turbidity occured, the weighed amounts gradually decreasing with the increase of the quantities of sulfur added.

The apparent specific gravity decreased with the increase of sulfate formation, being 9.3 per cent less for culture number 22 as compared with culture number 12.

C. Soil number 17.

Although sulfur oxidation did not seem to be greater in soil number 17 than in soil number 16, a number of cultures were neutral towards phenolphtalein at the end of the incubation period. The former soil having been leached, is considerably less alkaline and this counts for the fact that much less inoculated sulfur was necessary to produce neutrality or even acidity after such a comparetively short incubation period. The remaining alkalinity in culture numbers 29 to 31 after 12 weeks were bi-carbonates, and with the advance of sulfur oxidation the cultures would gradually become neutral to phenolphtalein.

The turbidity measurements indicate that the physical structure of the soil had been materially changed through the addition of inoculated sulfur.

The water holding power of this soil seemed also to be changed, and the apparent specific gravity was decreased to a considerable extent, showing that there had been a formation of aggregates as a result of the sulfur oxidation. The changes under discussion are recorded in table 22.

TABLE 22

Formation of sulfates and bi-carbonates, changes in total alkalinity, flocculation, turbidity, water holding capacity and apparent specific gravity in soil No. 17 after 12 weeks of incubation.

Culture Number	Pounds of Sulfur per acre	Milli-grams sulfate	Bi-carbonates	Total Alkalinity cc 0.1 N H_2SO_4	Transparent liquid in cc.	Turbidity (mgr) (salts included)	Water holding capacity	Specific Gravity
23	None	7.65#	1.00#	3.52#	0.5	98.0#	27.2	124.62
24	100	9.11	1.76	3.02	1.5	94.1	27.4	123.92
25	300	13.42	0.72	2.40	3.0	87.2	27.1	121.40
26	500	16.10	0.92	1.80	1.5	79.0	29.2	121.20
27	1000	17.19	1.02	1.50	2.5	71.3	27.8	118.70
28	1500	20.27	1.02	1.20	5.0	65.1	28.5	118.33
29	2000	23.37	0.40	0.40	8.0	59.2	32.0	116.15
30	2250	34.59	0.40	0.40	16.2	54.0	33.3	116.34
31	2500	39.10	0.20	0.21	31.1	52.0	33.2	113.27
32	3000	42.06	0.00	0.00	47.2	43.2	34.9	114.61
33	3500	43.48	Acid	Acid	48.1	37.1	32.0	113.84

Per 10 grams of soil

The formation of sulfates may be directly responsible for the forming of aggregates, but it is likely that the changes in the physical structure of all three soil under discussion is caused by the interrelated factors, as the changes in microbiological flora, sulfate formation, conversion of carbonates into bicarbonates, and a possible interchange of bases inthe soil.

The interdependence of the different phenomona recorded in the tables is brought out very strikingly in the graphs of fig.7 . The curves representing sulfate formation are inverted to show the relation more clearly.

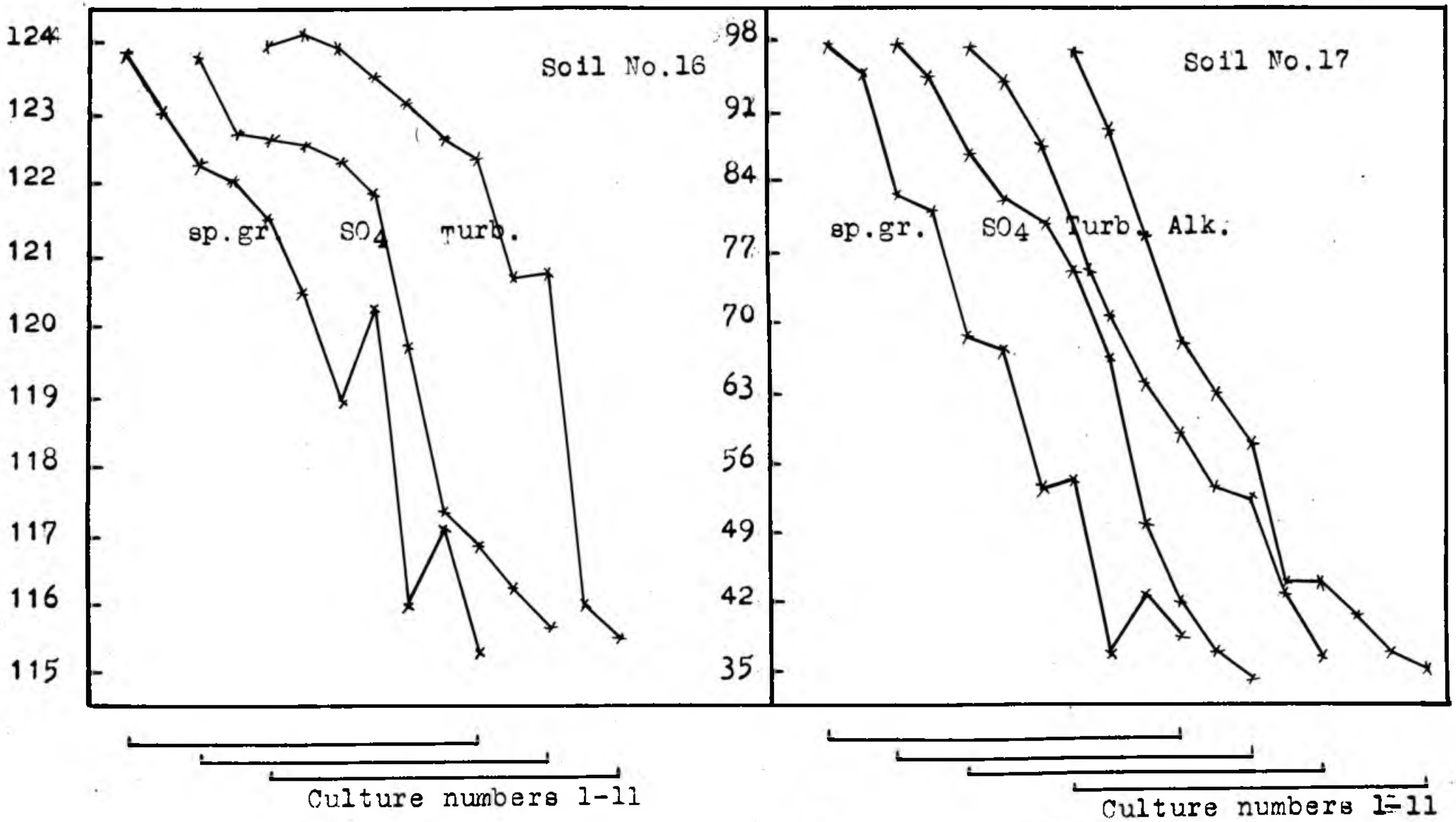


Fig.7.Curves represent apparent specific gravity, sulfates formed, turbidity, and changes in alkalinity of soils number 16 and 17 after an incubation period of 12 weeks.

The exact quantities and percentage of inoculated sulfur oxidized, as far as they are changed into the water soluble form, at the end of the incubation period of 12 weeks for the different soils under discussion, are given in table 23.

In several instances sulfur oxidation in soils numbers 16 and 17 had apparently been greater than in soil number 1, especially in the cultures to with larger amounts of inoculated sulfur had been applied. It may be that in some instances not all soluble sulfates were removed by the shaking or that small amounts of calcium sulfate, which are practically insoluble in distilled water, were formed. It can be seen from this table that but from 50 to 60 per cent of the larger quantities of sulfur added had been oxidized in soil number 1, whereas about 70 to 80 per cent of sulfur was oxidized in soil number 17 to which the same quantities of sulfur were added.

Nearly all of the small amounts of sulfur added had been oxidized in all cultures.

If the hydrogen-ion concentration of soil number 17 is compared with the per cent of sulfur oxidized it is obvious that a close relation exists between

TABLE 23

Quantities of inoculated sulfur oxidized at the end of the incubation period of 12 weeks. (Expressed per 100 grams soil)

Culture Number.	Soil No.	1		16		17	
	Milligrams Sulfur added	Mgr. sulfur oxidized	Percent oxidized	Mgr. sulfur oxidized	Percent oxidized	Mgr. sulfur oxidized	Percent oxidized
1	5	4.4	88.0	4.7	94.0	3.0	60.0
2	15	14.7	98.0	15.2	100.1	14.5	96.7
3	25	24.1	96.4	17.2	68.9	20.3	81.2
4	50	52.3	104.4	20.0	40.0	37.2	74.4
5	75	56.2	74.7	23.5	32.3	42.8	57.2
6	100	64.8	64.8	58.7	58.7	52.4	52.4
7	112	60.0	53.6	86.9	75.7	89.8	79.1
8	125	77.1	61.7	95.6	76.4	103.8	81.5
9	150	79.3	52.7	97.7	65.1	113.7	75.8
10	175	89.1	50.8	108.9	62.2	119.4	68.2

the readings of the pH values and the amounts of sulfur oxidized. The neutral point was reached after 81.5 per cent of the sulfur added to culture number 8 was oxidized, being approximately the calculated amount of sulfur necessary to neutralize the alkalinity of the soil. The cultures to which a greater surplus of sulfur was added and in which more than the theoretical amounts necessary wre oxidized, showed acidity, although this acidity by the titration method hardly could be detected.

In soil number 16 alkalinity still remained in all cultures, but the percentage of sulfur oxidized had also a great influence on the changes of the hydrogen-ion concentration. It seems that in all cases in these three soils the rate of oxidation was not entirely dependent upon the amounts of sulfur added, although the larger quantities naturally changed the high alkalinity more and at a higher rate.

CONCLUSIONS.

A. Handford sandy loam.

1. Small amounts of inoculated sulfur had little or no influence on the change of the hydrogen-ion concentration of soil number 1,which had received fertilizers for about 30 years,but additions of larger quantities lowered the pH values.
2. The influence of sulfur oxidation was greater in soil number 2,which had received no fertilizers for 33 years,and which was poorer in nitrogen an mineral plant food constituents tham was soil number 1.
3. Hydrogen-ion concentration increased nearly proportional to the sulfur application.
4. Soils receiving rock phosphate in addition to the sulfur had usually a higher H-ion concentration than the soils in the pots receiving sulfur alone.
5. Soy bean plants,grown in soil treated with inoculated sulfur,were stimulated by small additions of sulfur,but were injured by larger additions.
6. Sulfur with acid phosphate in addition produced best soy bean plants,while the series receiving rock phosphate in addition to sulfur were poorest.
7. The root systems of these soy bean plants were stimulated by small quantities of sulfur added,but

depressed by larger amounts.

8. Nodule formation seemed to be stimulated with small amounts of sulfur, but decreased numerically with the increase of the quantities of sulfur applied.

9. The phosphorus of Florida soft rock phosphate becomes available when a point in hydrogen-ion concentration between pH values 3.1 to 2.8 is reached.

10. The acidity produced by the oxidation of sulfur in these soils was not sufficient to render phosphates more available, although the acidity produced was harmful to the soy bean plants.

11. It is shown that in sand cultures, if sufficient acidity is produced to make the phosphorus available, the plants are killed.

12. Doubt is expressed whethter greater beneficial effects can be expected from rock phosphate and inoculated sulfur mixtures, which have not been composted long enough previous to the application so that the sulfates and the free acid formed have reacted with the phosphorus of the rock phosphate, unless the soil is in need of sulfur.

13. The hydrogen-ion concentration became greater in the uncropped soil with sulfur additions, untill a certain point was reached; from then on the movement

was back towards the neutral point.

14. The biological flora, expsessed in numbers counted on agar plates from soil infusions, was slightly stimulated by small sulfur applications, but depressed with larger amounts.

15. The formation of sulfates was influenced by the water content of the soil.

16. The soil to which inoculated sulfur is added seems to assume a new set of physical properties through the formation of sulfates, characterized by a more complete flocculation, change in water holding power and apparent specific gravity.

B. Fresno black alkali soil.

1. Small amounts of sulfur exerted little or no influence upon the hydrogen-ion concentration, but higher amounts brought about a decided change. The shanges were similar in cultures kept in an incubator at 28°C. and in cultures kept in pots in the greenhouse.

2. The leached soil was more readily neutralized by the addition of inoculated sulfur than the unleached soil, as indicated by titration and hydroegn-ion concentration methods.

3. Carbonates were transformed into bi-carbonates with the increase in sulfur oxidation. Although high alkalinity was changed into less alkalinity, high salt contents remained since no leaching occured.

4. The biological flora expressed in number of colonies grown on agar plates varied directly with the change in hydrogen-ion concentration. The cultures with the unleached soil, which received sufficient amounts of inoculated sulfur to neutralize the alkalinity, produced after 12 weeks of incubation, 5 times as many colonies per plate as did the untreated cultures, and the treated cultures of the leached soil from 3 to 5 times as many colonies as the untreated cultures.

5. The biological flora changed with the increase oß the hydrogen-ion concentration. In the leached soil cultures, which reached or appoached the neutral point aftar sulfuz application, there were but relatively few colonies of molds and actinomyces, whereas the colonies counted

on agar plates made from infusions of untreated soil, consisted largely or nearly entirely of molds and actinomyces.

6. There was a striking relation between the sulfate formation and the deflocculation, turbidity and the apparent specific gravity of the leached and unleached soils. Sulfate formation tends to cause aggregation of the soil, and the water holding power seems to be changed.

7. Barley seeds germinated and grew readily in pots treated with inoculated sulfur, but the plantlets were frequently killed by the salt incrustations on top of the soil, which incrustations were dissolved when the pots were watered, but could not penetrate the soils on account of their poor physical condition.

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