INFLUENCE OF SULFUR OXIDATION UPON GROWTH OF SOY BEANS AND ITS EFFECT ON BACTERIAL FLORA OF SOIL¹

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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 mechanism 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 review 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 (6) studying the fertilizer action of sulfur found that but little sulfur was oxidized in a sterilized garden soil, and reached the same conclusions. Bernhard (2) states that the beneficial effect of sulfur for the control 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 (11) have shown that elemental sulfur is oxidized by the proper bacteria. Later studies at the New Jersey Experiment Stations, have confirmed the earlier experiments. Martin (13) recently published results obtained with inoculated and uninoculated sulfur for the control of potato scab, which show that inoculated sulfur produced greater amounts of soil acidity than did uninoculated sulfur, and was superior for the control of potato scab.

There has been, and still is, considerable controversy as to the necessity of applying sulfur to the soil for the stimulation of plant growth. Lyon and Bizzel (12) 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 (21) state that the loss of sulfur due to the amount taken up by crop is insignificant as compared with the total amount which has disappeared from the soil. Hart and Peterson (8) calculate the loss of sulfur in drainage water to be three times the amount brought down to an acre from the atmosphere. Stewart (20) concludes that under humid conditions sulfur need not be added to the soil as plant food. Suffi-

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cient 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 (17) 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 (18), 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 superphosphate, gypsum, iron sulfate, etc. Headley (9, p. 16-18) 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 (14) 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 (23) 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 cruciferae on one soil and not on the other. Sulfur increased the yields of barley seed on a silt loam apparently 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 activities has been studied in several instances. Janicaud (10) states that sulfur has a favorable influence on the development of bacteria in the soil. Duley (7) 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, nitrification, 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 nitrifying bacteria. O'Gara (15) reporting field experiments with field and truck crops in which elemental sulfur was added at a rate of 400 pounds 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 (22) 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 (14) 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 legume bacteria; the number of nodules on the clover roots were increased. Pitz (16) 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 in ammonification, accompanied by a parallel decrease in nitrate formation.

EXPERIMENTS WITH SLIGHTLY ALKALINE SOILS

From the short review of literature it seems clear that some soils respond to the treatment of sulfur and others do not. The study reported below was primarily undertaken to determine whether or not a slightly alkaline soil, which produces chlorosis, would be benefited 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 soy beans.

The soil used was a Hanford fine sandy loam, and secured by Prof. C. F. Shaw of the University of California from ranches near Pomona, Cal. Professor 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 1 was taken in an orange grove about 100 feet from the west line of the orchard. The orange 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 samples represent a good soil in which the fertility has been maintained reasonably well through fertilizing and through tillage.

Soil 2 was taken about 150 feet west of soil 1 and about 50 feet west of the boundary line between the orange grove and the adjoining corn field. The field from which soil 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. The ranch is run by a Mexican tenant and the system of culture is poor. 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 in corn this year with a fair stand.

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

TABLE 1
Mechanical analyses of Hanford fine sandy loam

GRADE OF SOIL	ORANGE GROVE	OPEN FIELD
	<i>per cent</i>	<i>per cent</i>
Fine gravel.....	2.85	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

Flowers of sulfur was inoculated with one per cent of soil which was known to contain sulfur-oxidizing organisms. This inoculated sulfur was then thoroughly mixed with soils 1 and 2 and the water-holding capacity determined. The soils were kept at 60 per cent of the water-holding capacity throughout the period of investigation.

Hydrogen-ion concentration determinations were made of the soil extract before and after mixing with sulfur and at definite intervals during the growth of the plants, using the apparatus described by Van Alstine (24).

The soils were divided into 2 parts, one to be used for plant cultures and the other for bacterial studies.

SERIES 1. VEGETATION EXPERIMENTS WITH SOIL 1

Soy beans were grown in earthenware pots filled with 5 kgm. 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. The exact amounts of sulfur added to all series were as follows:

CULTURE NUMBER	POUNDS 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 plantlets 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 influence of plant growth on the hydrogen-ion concentration was but slight. In the water extract of the soils treated with small amounts of sulfur the changes were not very marked, but the larger quantities of sulfur exerted a decided influence, although not as much as would 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 rock phosphate had also been added, had been very slightly affected, while the effect on the plants grown in the cultures receiving the same amounts of sulfur but acid phosphate instead of rock phosphate were more distinct. The yellow spots on the plants in the other cultures were at that time more pronounced than after 3 weeks. The soil used was at the beginning of the experiment slightly alkaline, or neutral, with pH values varying between 7.2 and 7.0.

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.

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 very few, but extremely large, nodules. Some of these nodules were $\frac{3}{4}$ -1 cm. in diameter.

Although the weight of the plants decreased toward 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 3 out of 6 plants harvested after 4 weeks, leaving the 3 best-looking plants in each pot. At this time the cotyledons of the check plants and of the plants receiving 1000 pounds of sulfur per acre were all still dark green, while most of the cotyledons 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.

It was 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 than the pots receiving acid phosphate in addition, and also higher than the pots which received 1000 pounds of sulfur alone. The hydrogen-ion concentration went up more or less in all cultures.

According to the notes taken, all plants were found blooming after 4½ weeks, while cultures 5, 10 and 15 had very yellow leaves of which some had been dropped at that time.

Results are given in table 2.

The results obtained were much more pronounced than those obtained with the first crop. 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 than those of the plants in the check pots, but the roots of the plants in pots with higher sulfur applications were considerably less extensive,

TABLE 2

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

POT NUMBER	DRY WEIGHT OF TOPS	RELATIVE WEIGHT	TRANSPIRATION AND EVAPORATION	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION	REMARKS
Treated with sulfur								
1 (check)	3.0302	100.0	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	21.0	2	6.6	6.3	
4	2.2741	75.1	3535	21.0	6	6.6	6.1	Slightly injured
5	2.0000	66.0	3305	21.5	5	6.2	6.0	Strongly injured, cotyledons green
Sulfur plus 300 pounds of rock phosphate								
6 (check)	3.2750	100.0	3875	21.5	13	7.0	6.7	
7	2.9348	89.6	3710	22.0	12	6.9	6.4	
8	2.8441	86.9	3755	23.0	10	6.8	6.2	
9	2.1642	66.2	3390	22.0	7	6.5	6.1	
10	1.3470	41.1	3080	19.0	0	6.1	4.6	Strongly injured, cotyledons green
Sulfur plus 100 pounds of acid phosphate								
11 (check)	3.0227	100.0	3695	24.0	8	7.0	6.7	
12	3.2712	108.1	4030	26.5	15	6.9	6.5	
13	2.5400	84.0	3480	23.0	7	6.8	6.3	
14	2.4882	82.5	3505	23.0	8	6.6	6.1	
15	1.9160	63.3	3235	21.5	3	6.2	5.8	Injured, green cotyledons

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 phosphate were not as great as for the plants without phosphate, 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 actual acidity of culture 5 was even much less than of culture 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.

VEGETATION EXPERIMENTS WITH SOIL 2

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

The changes in acidity were in this case quite similar to the changes in series I. After 3 weeks the plants were, in general, much poorer than the plants grown in series I. The leaves of nearly all plants had yellow spots, and some of them were more or less drooping. The general appearance and average height were considerably inferior after 6 weeks when 3 of the 6 plants from every culture were harvested, than was the case with plants grown in soil 1. The effect of sulfur was more pronounced and cultures receiving 1000 pounds of sulfur per acre were severely injured.

The soil responded far better to the treatment of phosphorus than the soil which had received fertilizers for the last 30 years. The cultures receiving 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, while the others produced less dry weight.

All plants were scored for general appearance, yellowness and stockiness before harvesting. The scores show even more clearly than the dry weights of the plants that all cultures receiving 100 pounds of sulfur were better looking, greener and less spindling 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 more definitely the same facts as were found in soil 1. The extent of the root system and also the number of nodules decreased with the increase of the sulfur applications, except on plants in cultures 2, 7, and 11, which were relatively greater.

Here again, a second crop of soy beans was planted in the same pots and all applications of sulfur, rock phosphate and acid phosphate were repeated.

The hydrogen-ion concentration in this second-crop experiment was considerably higher than in the case of the second crop on soil 1. The highest concentration was again found in culture 10, which received 300 pounds of rock phosphate to the acre besides 1000 pounds of sulfur. A number of the cultures were apparently less acid after 6 weeks than at the end of 5 weeks.

Notes taken a few days before harvesting show that the cotyledons 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 dark green cotyledons. Culture numbers 3, 8 and 13 seemed most mature, while culture numbers 5, 10 and 15 were severely injured.

The dry weights and other data secured from the second crop are given in table 3.

TABLE 3
Yields of tops of second crop of soy beans grown in soil receiving no fertilizers for more than 30 years

POT NUMBER	DRY WEIGHT OF TOPS	RELATIVE WEIGHT	TOTAL TRANSPI- RATION AND EVAPO- RATION	AVERAGE HEIGHT	NUM- BER OF PODS	INITIAL REAC- TION	FINAL REAC- TION	REMARKS
<i>Treated with sulfur</i>								
1	gm.		cc.	cm.		pH	pH	Two plants blooming, strongly injured
1	2.5990	100.0	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	
<i>Sulfur plus 300 pounds of rock phosphate</i>								
6	2.3855	100.0	3665	22.0	9	6.9	6.3	Strongly injured
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	
<i>Sulfur plus 100 pounds of acid phosphate</i>								
11	2.2160	100.0	3395	21.5	10	6.7	6.4	Medium injured
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	

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 be enough to make the rock phosphate available, although some of the injury to the plants can be attributed to the acidity. Still this does not explain the phenomena in full. It is very likely, therefore, that the relatively high amounts of sulfates formed were harmful 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 considerably. 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.

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 toward a certain degree of stimulation by small additions of sulfur, but depression when the acidity produced becomes too great.

EXPERIMENTS IN SAND CULTURES

The acidity produced by inoculated sulfur in the soil cultures seemed insufficient to attack the rock phosphate added.

To test out whether or not a similar phenomenon 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 nutrient solution (R^6C^2) as a basis.

Florida soft rock phosphate was substituted for the phosphorus of the nutrient solution. 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 the rate of 2 tons per acre which supplied approximately the calculated amount of phosphorus in the check (Shive's R^6C^2 solution) cultures.

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

The results obtained are given in table 4.

The inoculated sulfur with the rock phosphate 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 until 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. Adding inoculated sulfur only to Shive's nutrient solution

TABLE 4

Results of growing soy bean plants in sand cultures for 6 weeks with inoculated sulfur and rock phosphate, and Shive's R_5C_2 solution as a basis

CULTURE NUMBER	TREATMENT	WEIGHT OF TOPS	AVERAGE LENGTH	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION
		gm.	cm.		pH	pH
1	None	0.850	8	Bloom	5.8	6.8
3	Rock phosphate	1.454	11.5	Bloom	5.4	6.6
5	R_5C_2	3.610	15.0	4	5.3	6.6
6	R_5C_2 , rock phosphate	2.223	15.0	2	5.0	6.4
8	R_5C_2 , inoc. sulfur, rock phosphate	0.600	Dead		4.7	2.9
14	R_5C_2 , inoc. sulfur	0.811	Dead		5.2	4.3

had also a decided influence on the lowering of the pH value, but no such great differences occurred as in the cultures to which previously mixed inoculated sulfur was added. This could be expected since sulfur oxidation had already begun in the mixture. Nevertheless, the plants grown in all cultures were killed in a comparatively short time. In the cases where no sulfur was added the plants made the soil solution less acid, bringing it nearly to the neutral point. This corresponds with the experiences in culture solutions where a similar phenomenon takes place. The changes in hydrogen-ion concentration were regular as is shown by the pH determinations, which were made at definite intervals.

Apparently not sufficient acidity was produced in the soil cultures, since the acidity at which the phosphorus of the rock phosphate becomes soluble had not yet been reached. In the sand cultures this point was reached, but the plants were not able to survive. The fact that very little or no phosphorus is changed into a soluble form, has been found in the studies reported in another paper of

this series. To find the exact acidity necessary to change the Florida soft rock phosphate used in these experiments into soluble P^2O^5 , a curve was constructed from readings of pH values obtained by adding different amounts of 0.1 normal and normal sulfuric acid to the rock phosphate.

Ten grams of rock phosphate were shaken with definite amounts of sulfuric acid for 2 hours in a shaking machine and left standing overnight. An aliquot of the supernatant liquid was then drawn off and the hydrogen-ion

FIG. 1. GRAPH SHOWING THE HYDROGEN-ION CONCENTRATION AT WHICH ROCK PHOSPHATE BECOMES AVAILABLE

determinations made. At the critical point sufficient determinations were made to check up all points obtained. The results are graphically shown in figure 1.

The curve shows that the phosphorus 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 be 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 until 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.

BIOLOGICAL EXPERIMENTS WITH SOIL 1

In several instances it has been reported that sulfur and sulfates exerted a stimulating influence on bacterial growth. The possibly inadequate supply of sulfur in soils is apt to influence the microorganisms, 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 attention was paid to the occurrence and determination of different species.

The amounts of sulfur added are given in table 5. During the incubation period hydrogen-ion concentration determinations of the soil water extract were made at frequent intervals. It was found that in all cases the hydrogen-ion concentration became greater until a certain point was reached, from then on the movement was back toward the neutral point. This turning point was reached in all cases before or between the ninth and the eleventh 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 certain equilibrium. This phenomenon is even more clearly demonstrated by the curves shown in figure 2.

Bacterial numbers were determined by the plate method, using Lipman and Brown's synthetic agar, at the beginning of the experiment and after 6, 12 and 18 weeks. The numbers in thousands per gram of soil are given in table 5.

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

FIG. 2. CURVES SHOWING CHANGES OF HYDROGEN-ION CONCENTRATIONS OF SOIL 1 TREATED WITH DIFFERENT AMOUNTS OF INOCULATED SULFUR

TABLE 5
Bacterial numbers in soil 1 treated with inoculated sulfur

CULTURE NUMBER	SULFUR ADDED PER ACRE	NUMBER OF BACTERIA PER GRAM OF SOIL			
		Initial	After 6 weeks	After 12 weeks	After 18 weeks
	<i>lbs.</i>	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>
1	None	3.280	3.195	3.200	3.150
2	100	3.240	2.520	3.525	3.475
3	300	3.190	3.470	3.615	3.535
4	500	3.200	4.470	3.325	3.225
5	1000	3.195	4.160	3.165	3.100
6	1500	3.260	3.600	2.720	2.950
7	2000	3.240	2.675	1.840	1.950
8	2250	3.275	2.390	1.415	0.850
9	2500	3.260	1.935	1.345	0.550
10	3000	3.235	1.405	0.765	0.450
11	3500	3.250	1.390	0.560	0.350

amounts of acidity produced, the more sulfates there were formed, the fewer colonies were counted 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 original numbers. There is no doubt, however, that the depression of the original 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 cannot 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 greater for all cultures which received the higher applications, but the movement of the hydrogen-ion concentration was still farther toward 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.

After 24 weeks the majority of the cultures had reached the neutral point.

SUMMARY

Small amounts of inoculated sulfur had little or no influence upon the change of the hydrogen-ion concentration of soil 1, which had received fertilizers for about 30 years, but additions of larger quantities lowered the pH values.

The influence of sulfur oxidation was greater in soil 2, which had received no fertilizers for 33 years, and which was poorer in nitrogen and mineral plant food constituents than was soil 1.

Hydrogen-ion concentration increased nearly proportionally to the sulfur application.

Soils receiving rock phosphate in addition to the sulfur had usually higher hydrogen-ion concentrations than the soils in the pots receiving sulfur alone.

Soy bean plants, grown in soil treated with inoculated sulfur, were stimulated by small additions of sulfur, but were injured by larger additions.

Sulfur with acid phosphate in addition produced best soy bean plants, while the series receiving rock phosphate in addition were poorest.

The root systems of these soy bean plants were stimulated by small quantities of sulfur, but depressed by larger amounts.

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

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

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

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

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

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

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

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

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FIG. 1. SOY BEAN PLANTS GROWN IN SOIL WHICH HAD NOT RECEIVED FERTILIZERS FOR 30 YEARS. SULFUR ALONE ADDED. NO. 1 IS CHECK

FIG. 2. SOY BEANS PLANTS GROWN IN SOIL WHICH HAD BEEN FERTILIZED FOR MORE THAN 30 YEARS TO WHICH SULFUR ALONE WAS ADDED. NO. 1 IS CHECK