STUDIES IN SULFOFICATION.¹

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I.

Recent experiments (1) have shown that sulfofication or sulfur-oxidation is an important process occurring in field soils. Plants have been found to require considerable amounts of sulfates for their best growth and inasmuch as sulfur is not present in soils in that form but as unassimilable organic and inorganic compounds, it is apparent that the ability of a soil to produce sulfates from these unavailable substances will determine very largely the sulfur-feeding of the crops grown. In other words, the total sulfur content, alone, of a soil will not show the sulfur available for plant growth. The sulfofying or sulfate-producing power of the soil must also be ascertained.

The investigations mentioned besides demonstrating the fact that all soils possess a definite sulfofying power which is determinable in the laboratory, threw considerable light upon the conditions governing the process. Thus it was found that additions of green manure and barnyard manure increased the sulfofying power of the soil, and in general, that the treatment which the soil had undergone influenced considerably its ability to produce sulfates. Furthermore, the optimum moisture content of the soil for the occurrence of the process was found to be 50 per cent of the amount necessary for complete saturation, and the oxidation of sulfur was found to occur to the greatest extent in a mixture of 50 per cent soil and 50 per cent sand, showing the importance of aeration. Again the addition of carbohydrates to the soil was shown to depress sulfofication, the greater the amount added, the greater the depression, and the depression also varied in the inverse ratio to the solubility of the carbohydrates.

A definite laboratory method was devised for determining the sulfofying power of soils and this consisted in the addition of 0.1 gm. of Na_2S or free sulfur, preferably the latter, to 100-gm. quantities of fresh soil, adjustment of the moisture content to the optimum for the soil, and incubation for from 5 to 10 days. The sulfates were then determined by shaking the soil with water for 7 hours in the shaking machine, filtering, precipitating the sulfates with barium chloride, and estimating in the sulfur photometer.

¹ Received for publication March 10, 1916.

Studies on the sulfur content of Iowa soils confirm the observations of Hart and Peterson (2) in Wisconsin and Shedd (3) in Kentucky, that on the average much less sulfur than phosphorus was present in the various large soil areas. Some of the sulfur removed from the soil by crops may, of course, be returned by the use of manure, but the amount of manure produced on a livestock farm is quite insufficient, as a general rule, to keep up the sulfur content of the soil, and unless manure is purchased or large amounts of commercial feeds are used, commercial sulfur-containing fertilizers must be applied to maintain the soils permanently fertile.

This does not mean that applications of sulfur fertilizers would prove profitable on Iowa soils at the *present time*, but it does mean that unless different methods of soil treatment are employed than those at present in use, at *some future time*, sulfur will be lacking. In other words for *permanent soil fertility*, the sulfur supply for crops must be considered.

It is known at the present time that the amount of phosphorus present in Iowa soils is low, and in many cases this element may be the limiting factor of growth. Acid phosphate and rock phosphate are the two materials which are available commercially for supplying phosphorus. The former supplies sulfur as well as phosphorus and the question arises whether it may have any superior value because of the sulfur added. The relative merits of the two phosphorus compounds are not yet definitely known, and it is possible that the sulfur content, and also the effect on sulfification, should be considered in selecting the material which should be recommended for remedying deficiencies of phosphorus in soils.

The relative effects of the materials mentioned on sulfification, on ammonification, and on crop yields should also be ascertained. If sulfification and ammonification run parallel, it will be evident that methods of treatment which are of value because of a stimulation of nitrate production will also lead to greater sulfate production. If crop yields and sulfification are similarly affected, it may be that the effects of the materials are largely due to the sulfur factor.

Therefore, the following experiment was planned to throw some light upon the problem of the relative effects of gypsum, acid phosphate, rock phosphate, alone and with gypsum, and mono-calcium phosphate on sulfification, on ammonification and on the yields of oats in pots in the greenhouse.

THE PLAN OF THE EXPERIMENT.

The soil used in this work was a Carrington Loam, high in organic matter and having a basic reaction. When analyzed, it was found to contain 911 pounds of phosphorous and 2487 pounds of sulfur per acre of two million pounds of surface soil. This sulfur content was very high, much higher than that of any of the samples of Iowa soils whose analyses were given in the bulletin already referred to.

The results secured were undoubtedly modified considerably because of the presence of so much sulfur in the soil used. The use of gypsum for instance could hardly be expected to show any effect and all of the applications would probably exert much more influence on sulfofication in a soil lower in sulfur.

This soil was evidently somewhat abnormal for Iowa conditions, and hence the results should not be interpreted as of more than technical and special interest.

Twenty-four 4-gallon pots were filled with the soil described which was in an air-dried condition, 27.6 pounds being placed in each pot.

The special treatments were as follows:

<i>Pots.</i>	<i>Treatment.</i>
1- 2.	Check.
3- 4.	24.7 lbs. calcium sulfate per acre.
5- 6.	70.5 lbs. mono-calcium phosphate per acre.
7- 8.	300 lbs. acid phosphate per acre.
9-10.	1000 lbs. rock phosphate per acre.
11-12.	1000 lbs. rock phosphate + 24.7 lbs. CaSO_4 per acre.
13-14.	Check.
15-16.	24.7 lbs. CaSO_4 per acre.
17-18.	70.5 lbs. $\text{CaH}_4(\text{PO}_4)_2$ per acre.
19-20.	300 lbs. acid phosphate per acre.
21-22.	1000 lbs. rock phosphate per acre.
23-24.	1000 lbs. rock phosphate + 24.7 lbs. CaSO_4 per acre.

Pots 13 to 24 were seeded to oats and the remainder were kept bare for bacteriological tests.

The applications were based on actual field conditions, the 300 pounds of acid phosphate and 1000 pounds of rock phosphate forming the basis of the additions. The acid phosphate was analyzed for phosphorus and sulfur and showed a content of 5.2 per cent of phosphorus and 1.994 per cent of sulfur. The applications of calcium sulfate and mono-calcium phosphate made to the soil were calculated, so that the same amounts of sulfur and phosphorus, respectively, as were applied in the acid phosphate should be added.

The rock phosphate contained 5.85 per cent of phosphorus and hence considerably more phosphorus was applied than in the case of the acid phosphate, but the amounts of both materials used were those commonly employed on the farm, and hence a fair comparison was provided. The variation in available phosphorus, of course, accounts for the difference in the amounts applied.

The optimum moisture content of the soil was determined and after the pots were filled, distilled water was applied to bring all the soils up to

that content. The pots were then weighed and during the continuance of the experiment the moisture content was maintained by additions of distilled water to weight.

The oats were harvested just prior to maturity, the green and dry weights secured and the nitrogen content determined.

Samples were drawn for bacteriological tests every two weeks, the sulfates present were determined, the moisture content ascertained and tests for sulfofying power by the free-sulfur-fresh-soil method, previously described, and for ammonification by the casein-fresh-soil method and the dried-blood-fresh-soil method were carried out.

The usual precautions were observed in sampling to secure uncontaminated samples. The sulfate determinations were made by shaking the soil with water for 7 hours in the shaking machines as usual. The ammonia determinations were made by the magnesium-oxide method.

The experiment was begun on January 11th, and the samples were drawn on January 26th, February 9th, February 23rd, March 9th and March 23rd.

THE SULFATES PRESENT AT SAMPLING.

The amounts of sulfates present in the soils at the various samplings are given in Table I, and the average contents under the different treatments are calculated.

On examining the table it appears that there was little variation in sulfate content at the different samplings. The amounts added were very small and evidently the method used in the subsequent determinations was not sufficiently accurate to show them.

There are some indications in all the samplings, except the last, of a depression in sulfate content in the treated soils, but the differences were too small to be distinctive. The later sulfofying tests showed increases in sulfofying power, due to treatment, and hence it would hardly be reasonable to assume any depression in sulfate content in this case. The variations in results should, therefore, probably be regarded as due to the method of determination and as indicating the absence of any effect of the materials added, rather than as distinctive differences.

It is apparent, however, that the variations in sulfate content from one sampling to the next were very slight, much smaller than is usually the case with nitrates. There are such variations, however, that the conclusion seems justified that sulfate production and assimilation vary in much the same way that nitrate production and assimilation vary. That is, there may be an accumulation up to a certain point which is followed by increased assimilation and hence a decrease in the amount present. In the field, of course, there are losses in sulfates by leaching and assimilation by plants, just as in the case of nitrates, but in these experiments there was no leaching and no plants grown and hence the differences

were due to variations in production and assimilation by bacteria. There are evidently certain sulfate-assimilating bacteria which may become very active in the presence of abundance of sulfates and whose activity declines as the amounts of sulfates present are used up.

It will be left for future experiments to learn more of these sulfate-assimilating bacteria. Their activities may be a source of removal of sulfates from the use of crops, but it is more probable that they would be regarded as a means of preserving sulfates in the soil and preventing the loss by leaching. Sulfates which are used by the assimilating bacteria would later become available again for plant growth, and hence at times of too large sulfate production for the needs of crops. These bacteria would prove of much value in preventing losses by leaching.

TABLE I.
SULFUR AS SULFATES PRESENT AT TIME OF SAMPLING.

Pot No.	Treatment	Lab No.	1st Samples		2nd Samples		3rd Samples		4th Samples		5th Samples	
			Mg. S. as SO ₄	Av. for Treatment	Mg. S. as SO ₄	Av. for Treatment	Mg. S. as SO ₄	Av. for Treatment	Mg. S. as SO ₄	Av. for Treatment	Mg. S. as SO ₄	Av. for Treatment
1	Check	1	4.98		6.22		5.95		5.20		5.36	
		2	5.07		7.27		5.81		5.71		4.48	
2	Check	3	4.88		7.27		6.87		6.53		6.68	
		4	5.17	5.02	7.27	7.00	7.28	6.50	6.29	5.93	6.52	5.75
3	CaSO ₄	5	3.91		5.81		6.36		5.88		5.84	
		6	4.75		5.61		6.36		6.12		6.92	
4	CaSO ₄	7	3.87		5.40		5.61		5.88		5.36	
		8	5.17	4.43	5.71	5.64	5.71	6.01	5.61	5.87	5.52	5.91
5	CaH ₄ (PO ₄) ₂	9	4.83		6.36		7.21		6.12		6.84	
		10	4.83		7.82		7.14		6.46		6.56	
6	CaH ₄ (PO ₄) ₂	11	4.54		5.61		5.11		4.93		5.68	
		12	5.17	4.84	5.34	6.28	5.71	6.44	4.59	5.52	5.68	6.19
7	Acid Phos.	13	4.70		5.27		6.87		5.24		6.28	
		14	5.37		5.44		6.02		5.78		6.60	
8	Acid Phos.	15	4.83		4.56		5.34		4.83		6.12	
		16	5.17	5.02	4.83	5.02	5.27	5.89	5.24	5.27	6.33	6.33
9	Rock Phos.	17	3.64		4.25		4.56		4.69		5.52	
		18	4.39		4.18		4.49		4.69		5.12	
10	Rock Phos.	19	3.51		4.25		4.39		4.96		5.36	
		20	4.70	4.02	4.35	4.26	4.56	4.49	4.76	4.77	5.52	5.38
11	R'k Ph. + CaSO ₄	21	4.99		5.27		6.46		7.07		6.84	
		22	5.17		5.44		6.36		6.60		6.84	
12	R'k Ph. + CaSO ₄	23	3.79		5.40		6.53		5.20		5.76	
		24	4.88	4.71	5.40	5.37	6.38	6.43	5.44	6.07	6.08	6.38

It is evident also from these results that sulfates do not accumulate in soils any more than nitrates do. They seem to be subject to much the same influences as nitrates, and this fact suggests that sulfate production and also sulfate assimilation are very closely related, respectively, to nitrate production and nitrate assimilation, and that the influence of certain known factors on the nitrogen processes may be the same on the sulfur processes.

THE SULFOFICATION TESTS.

The samples drawn on the dates given previously, were tested for their sulfofying power according to the method described,—the free-sul-fur-fresh-soil method.

The results secured at the various samplings are given in Tables II, III, IV, V and VI and the average results for the different treatments appear in Table VII.

TABLE II.
PER CENT OF ADDED SULFUR, SULFOFIED, 1st SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO ₄	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ in soil after incubation	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ from S. added	% S. Sulfofied for each treatment
1	Check	1	lost		7.75			
		2	lost		7.36	7.55		
2	Check	3	38.76		8.46			
		4	36.72	36.74	8.23	8.33	28.40	28.40
3	CaSO ₄	5	43.69		7.47			
		6	42.84	43.26	7.47	7.47	35.79	
4	CaSO ₄	7	43.69		7.34			
		8	45.90	44.78	7.34	7.34	37.44	36.60
5	CaH ₄ (PO ₄) ₂	9	40.46		7.92			
		10	42.50	41.48	8.09	8.00	33.48	
6	CaH ₄ (PO ₄) ₂	11	48.62		7.27			
		12	41.48	45.05	7.41	7.34	37.71	35.60
7	Acid Phos.	13	42.50		7.75			
		14	41.99	42.24	7.75	7.75	34.49	
8	Acid Phos.	15	38.76		5.88			
		16	36.38	37.57	5.57	5.72	31.85	33.20
9	Rock Phos.	17	43.69		5.71			
		18	41.16	42.41	5.71	5.71	36.70	
10	Rock Phos.	19	32.64		5.27			
		20	32.64	32.64	5.44	5.35	29.29	33.00
11	R'k Ph. + CaSO ₄	21	27.06		7.27			
		22	36.38	36.72	7.58	7.42	29.30	
12	R'k Ph. + CaSO ₄	23	36.04		6.22			
		24	34.68	35.36	6.35	6.28	29.08	29.20

On examining the results given in the complete tables, it is found that the duplicate determinations agreed very closely, indicating that the method employed in the estimation of the sulfates was quite satisfactory.

The results from the duplicate pots were not always in perfect agreement, but that is ever the case in greenhouse experiments. Differences in the location of duplicate pots with reference to the glass, seem to exert an important influence on the bacteriological results as well as on the crop yields secured in the greenhouse. Of course, there is the danger of accidental contamination in soils under such abnormal conditions as pertain in the greenhouse, as indicated by the growth of algae, which is frequently observed, the occurrence of molds and possibly also of proto-

zoans. But, in general, the differences in the heat and light relations may account for many of the variations which are encountered.

The results secured in this work from the duplicate pots were as uniform as is usually the case and whatever the causes of the variations may be, it was impossible to ascertain them, and hence the average results must be considered as fairly accurate.

TABLE III.
PER CENT OF ADDED SULFUR, SULFOFIED, 2ND SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO ₄	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ in soil after incubation	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ from S. added	% S. Sulfofied for each treatment
1	Check	1	32.30		7.99			
		2	31.11	31.70	8.19	8.09	23.16	
2	Check	3	28.07		7.99			
		4	29.75	28.91	8.74	8.36	20.25	21.72
3	CaSO ₄	5	31.11		7.28			
		6	31.11	31.11	7.21	7.24	23.87	
4	CaSO ₄	7	35.19		6.97			
		8	36.89	36.04	6.87	6.92	29.12	26.50
5	CaH ₄ (PO ₄) ₂	9	35.19		8.02			
		10	35.53	35.36	8.40	8.21	27.15	
6	CaH ₄ (PO ₄) ₂	11	35.19		5.75			
		12	36.38	35.78	5.75	5.75	30.03	28.60
7	Acid Phos.	13	28.07		7.28			
		14	30.60	29.33	7.07	7.17	22.16	
8	Acid Phos.	15	28.90		6.29			
		16	26.69	27.79	5.88	6.08	21.71	21.90
9	Rock Phos.	17	30.09		5.34			
		18	31.11	30.60	5.20	5.22	25.38	
10	Rock Phos.	19	31.79		5.34			
		20	29.24	30.01	5.05	5.19	24.82	25.10
11	R'k Ph. + CaSO ₄	21	27.20		6.46			
		22	34.85	31.02	6.53	6.49	24.52	
12	R'k Ph. + CaSO ₄	23	27.71		6.73			
		24	31.79	29.75	6.29	6.50	23.25	23.90

Each table gives the amounts of sulfates present in the soils after incubation, and upon examining these results and comparing them with the amounts of sulfates which the soils contained at sampling, given in Table I, it appears that the incubation of the soils for 10 days brought about only very slight changes in the sulfate content of the soils. It is evident, therefore, that the amount of sulfates present in soils does not change to any great extent in short periods of time. In other words in the absence of leaching and of assimilation by crops there seems to be somewhat of an equilibrium established between sulfate-production and sulfate-assimilation. At any rate, the sulfate content of soils under these conditions changes so slowly that tests made within short intervals of time do not seem to show any large differences.

Under field conditions it is quite probable that the differences would be much greater and would appear in a much shorter space of time. In short, it seems extremely doubtful if an equilibrium such as was found here would be established under field conditions in the presence of the disturbing factors of leaching and assimilation by crops. Unless special treatments were followed it would be reasonable to expect that the sulfate content of soils would gradually decline, and such is actually the case in the field. As the total sulfur content becomes less the production of sulfates becomes slower, as has been shown in the sulfonation studies already referred to. Hence under field conditions instead of an equilibrium in sulfates, a gradual decline is found unless special treatments are followed.

TABLE IV.
PER CENT OF ADDED SULFUR, SULFOFIED, 3RD SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO ₄	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ in soil after incubation	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ from S. added	% S. Sulfofied treatment for each
1	Check	1	33.66		6.02			
		2	33.32	33.49	5.88	5.95	27.54	
2	Check	3	34.85		6.60			
		4	34.85	34.85	6.60	6.60	28.25	27.90
3	CaSO ₄	5	39.10		7.28			
		6	39.61	39.35	6.97	7.12	32.23	
4	CaSO ₄	7	43.69		5.71			
		8	35.70	39.65	5.88	5.79	33.86	33.00
5	CaH ₄ (PO ₄) ₂	9	34.34		7.44			
		10	35.36	34.85	7.28	7.36	27.49	
6	CaH ₄ (PO ₄) ₂	11	31.79		5.17			
		12	33.66	32.72	5.34	5.25	27.47	27.50
7	Acid Phos.	13	32.30		5.20			
		14	31.96	32.13	4.42	4.81	27.32	
8	Acid Phos.	15	34.34		4.69			
		16	33.66	34.00	5.13	4.91	29.09	28.20
9	Rock Phos.	17	37.23		4.79			
		18	35.36	36.29	4.69	4.74	31.55	
10	Rock Phos.	19	41.99		5.00			
		20	38.76	40.37	4.83	4.91	35.46	33.50
11	R'k Ph. + CaSO ₄	21	39.95		5.85			
		22	41.99	40.92	6.19	6.02	34.96	
12	R'k Ph. + CaSO ₄	23	32.30		5.95			
		24	34.00	30.15	5.95	5.95	27.20	31.00

Upon subtracting the sulfate content of the soils after incubation from the total amount of sulfates found in the tests, the remainder is calculated as per cent of sulfur sulfofied, and these are the figures which show the sulfofying power of the soils.

Turning to Table VII which gives the average percentages of sulfur sulfofied, some interesting facts become evident.

In the first place it is found that the calcium sulfate, even in the small applications made, increased to a marked degree the sulfofying power of the soil. This marked increase occurred at every date of sampling, and bears out the results secured in the earlier experiments already referred to, according to which calcium sulfate in various amounts increased the sulfofying power of the soil used to a large extent, the influence being in direct proportion to the size of the application. Of course, if the amount of sulfate applied were increased beyond a certain point it is probable that no further increase in sulfofication would occur and an actual depression might take place. The interesting feature of the present results is that very small amounts of gypsum, such as may be added to soils in another fertilizing material (acid phosphate), may exert a pronounced influence upon the ability of the soil to produce sulfates.

TABLE V.
PER CENT OF ADDED SULFUR, SULFOFIED, 4TH SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO ₄	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ in soil after incubation	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ from S. added	% S. Sulfofied for each treatment
1	Check	1	34.6		6.92			
		2	34.2	34.4	6.92	6.92	27.5	
2	Check	3	33.6		5.48			
		4	34.4	34.0	5.76	5.62	28.4	27.9
3	CaSO ₄	5	31.6		6.20			
		6	32.0	31.8	6.40	6.30	25.5	
4	CaSO ₄	7	41.6		6.32			
		8	45.6	43.6	6.04	6.18	37.4	31.4
5	CaH ₄ (PO ₄) ₂	9	52.2		5.12			
		10	50.0	51.1	5.76	5.44	45.6	
6	CaH ₄ (PO ₄) ₂	11	43.2		5.08			
		12	44.2	43.7	4.96	5.02	38.7	42.1
7	Acid Phos.	13	48.8		6.88			
		14	46.6	47.7	6.84	6.86	40.8	
8	Acid Phos.	15	47.0		6.72			
		16	46.0	46.5	6.32	6.52	40.0	40.4
9	Rock Phos.	17	50.0		5.36			
		18	51.4	50.7	5.16	5.26	45.5	
10	Rock Phos.	19	47.0		5.40			
		20	43.8	45.4	5.40	5.40	40.0	42.7
11	R'k Ph. + CaSO ₄	21	35.0		7.32			
		22	42.0	39.5	6.92	7.12	32.4	
12	R'k Ph. + CaSO ₄	23	40.4		6.40			
		24	39.2	39.8	5.40	5.90	33.9	33.1

In other words, the effects of gypsum may be partly due to a stimulative action as has been supposed as well as to the addition of a plant food constituent. The stimulative action may be of considerable importance on soils which contain sufficient amounts of total sulfur but do not have a rapid enough sulfofying action. In other words if soils are found which

contain fairly large amounts of sulfur but on which crops are not supplied with sufficient sulfates for their best growth, applications of small amounts of gypsum might be sufficient to stimulate sulfofication to such an extent that the sulfur already present in the soil would be sulfofied rapidly enough to keep plants supplied with that element.

The mono-calcium phosphate gave considerable increases in sulfofication, and these were especially pronounced at the last two samplings. The increases were very similar to that brought about by the gypsum, varying somewhat from those results, as might be expected. It is apparent that this material exerted some stimulative action on sulfofication, and if it has any effect on crop growth, that effect might be considered to be due

TABLE VI.
PER CENT OF ADDED SULFUR, SULFOFIED, 5TH SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO ₄	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ in soil after incubation	Av. Mg. S. as SO ₄	Mg. S. as SO ₄ from S. added	% S. Sulfofied for each treatment
1	Check	1	35.4		5.12			
		2	36.6	35.9	5.08	5.10	30.8	
2	Check	3	45.6		5.72			
		4	44.6	45.1	5.72	5.72	29.4	30.1
3	CaSO ₄	5	36.6		5.88			
		6	47.6	48.1	6.16	6.02	41.1	
4	CaSO ₄	7	45.6		5.40			
		8	44.6	45.1	5.40	5.40	39.7	40.4
5	CaH ₄ (PO ₄) ₂	9	45.6		5.88			
		10	43.2	44.4	5.88	5.88	38.6	
6	CaH ₄ (PO ₄) ₂	11	50.0		5.00			
		12	47.6	48.8	4.96	4.98	43.8	41.1
7	Acid Phos.	13	40.4		6.24			
		14	40.4	40.4	6.04	6.14	34.3	
8	Acid Phos.	15	48.8		5.68			
		16	45.6	47.2	5.56	5.62	41.6	37.9
9	Rock Phos.	17	47.0		5.52			
		18	46.6	46.8	5.16	5.34	41.5	
10	Rock Phos.	19	45.6		5.16			
		20	43.8	44.7	5.04	5.10	39.6	40.5
11	R'k Ph. + CaSO ₄	21	45.6		7.68			
		22	47.6	46.6	6.44	7.06	39.5	
12	R'k Ph. + CaSO ₄	23	46.0		6.04			
		24	49.4	47.7	6.04	6.04	41.7	40.6

in part to an increased production of sulfates and not entirely to the phosphorus supplied. The action of this material may be somewhat indicative of the effect of acid phosphate, assuming that the phosphorus in this latter material is in the mono-calcic form which it is in part at least.

The applications of acid phosphate increased the sulfofying power of the soil, but to a smaller extent in practically all cases than either the gypsum or the mono-calcium phosphate alone. It appears, therefore, that

on this soil the combination of the two substances was not as beneficial for sulfofication as either of them alone. Just why this should be the case is difficult to determine. It is probably, however, the result of more complicated bacterial changes brought about by the combined substances, although the other calcium phosphates present in the acid phosphate, such as the dicalcic and tricalcic phosphates may explain the different effects.

An interesting practical point is brought out here, however. The acid phosphate when applied to this soil had a stimulative action on sulfofication, and hence its influence on crop yields, if it exerts any effect whatever, may not be due entirely to the phosphorus which it supplies to the crops in available form or to the sulfate which is supplied, but in part to the increase in sulfate production from the soil. Previous suggestions regarding the value of acid phosphate because of effects on the sulfur feeding of plants are thus confirmed, and it seems reasonable to conclude that on soils deficient in both phosphorus and sulfur, acid phosphate would be a good material to use to supply both deficiencies, increasing the sulfates available for plants both by actual additions and by increased production in the soil.

TABLE VII.
PER CENT OF ADDED SULFUR, SULFOFIED FOR EACH TREATMENT
AT EACH SAMPLING.

Treatment	Samples				
	1st	2nd	3rd	4th	5th
1. Checks	28.4	21.7	27.9	27.9	30.1
2. 24.7 lbs. CaSO_4	36.6	26.5	33.0	31.4	40.4
3. 70.5 lbs. $\text{CaH}_4(\text{PO}_4)_2$	35.6	28.6	27.5	42.1	41.1
4. 300 lbs. Acid Phosphate	33.2	21.9	28.2	40.4	37.9
5. 1000 lbs. Raw Rock Phosphate	33.0	25.1	33.5	42.7	40.5
6. 1000 lbs. Raw Rock Phosphate plus 24.7 lbs. CaSO_4	29.2	23.9	31.0	33.1	40.6

Raw rock phosphate applied at the rate of 1000 pounds to the acre, a normal farm application, increased the sulfofying power of the soil to a greater extent than did the acid phosphate also applied in the customary field amount. The increase was about the same as that exerted by the mono-calcium phosphate. Only one reason suggests itself in explanation of the greater influence of the rock phosphate over the acid phosphate—that it is due to the greater amount of phosphate used. Perhaps the sulfofying bacteria use phosphorus in their growth and the stimulative effect of phosphorus fertilizers on sulfofication is really due to a feeding of the sulfofiers. In such a case, which seems quite probable, the question arises as to in what form the phosphorus is required by the bacteria. Probably it must be in a soluble form when it would be expected that the acid phosphate would give greater increases than the rock phosphate.

It is interesting to consider this effect of rock phosphate on sulfification from the practical standpoint. If raw rock phosphate will stimulate sulfate production to as large an extent as these results indicate, it may be that the material would be quite as valuable as a phosphorus *and sulfur* fertilizer such as acid phosphate, at least on soils not extremely low in sulfur. In other words, if rock phosphate will stimulate sulfate production from soils sufficiently to supply the needs of crops, it may be unnecessary to use a special sulfur fertilizer except in extreme cases, and the phosphorus fertilizer may be depended on for a dual purpose. Of course, this is assuming that the rock phosphate gives as good effects from the phosphorus standpoint as the acid phosphate, a point which, as has been mentioned, is far from being settled at the present time.

When gypsum was applied with the rock phosphate, increases in sulfification were noted, but these gains were smaller than those secured with the rock phosphate alone and smaller also than those given by the gypsum alone. The increases were about the same as those given by the acid phosphate. It is apparent again, therefore, that the single constituents gave more effect than the two together. In this case, also, just as with the acid phosphate, the cause for this smaller increase with the combined materials is not apparent and may be due to complicated bacterial changes where the two substances were combined. It is evident that on soils not very deficient in total sulfur, rock phosphate alone may prove just as beneficial as when applied with gypsum because of a greater production of available sulfates.

It must be emphasized again that these results apply to this particular soil only and not to soils in general. The soil used in this work was unusually high in sulfur, as has been pointed out, and hence the effects of sulfur fertilizers would be less pronounced than on soils poorer in sulfur. If there are such pronounced effects on the sulfifying power of this particular soil by small applications of the various fertilizing materials, a much greater effect might be expected from the same substances on a soil poorer in sulfur, or a more normal soil.

Therefore, the following conclusions from this work seem entirely justified, and while they apply specifically to this particular soil, they may be found to be of much more general application:

Applications of acid phosphate, of rock phosphate, of gypsum, of rock phosphate and gypsum, and of mono-calcium phosphate increased the sulfifying power of the soil to a considerable extent.

The rock phosphate, mono-calcium phosphate and gypsum gave the largest increases, larger than those given by the mixtures or by the acid phosphate.

Any of these materials, therefore, when applied to the soil in normal field amounts may be expected to increase sulfate production. Their ef-

fects on crops grown, if any, may be due partly at least to this influence on sulfur transformation. It is particularly interesting to note the greater effect of the rock phosphate than of the acid phosphate on sulfofication. On soils not strongly depleted in sulfur, therefore, but deficient in sulfofication, and also in need of phosphorous, it seems possible that the rock phosphate would prove as satisfactory as the more soluble acid phosphate. Crop yields must, of course, prove this point before it can be accepted definitely.

No reason can be assigned for the greater effects on sulfofication of the single constituents over the combinations. They were probably due to complicated bacterial processes which the latter engendered, and about which nothing is known as yet.

THE AMMONIFICATION EXPERIMENTS.

The samples drawn on the dates already mentioned were tested for their ammonifying power by the casein-fresh-soil method and the dried-blood-fresh-soil method. The former method was employed at the first

TABLE VIII.
AMMONIFICATION TESTS.

Pot No.	Treatment	Lab No.	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment
1	Check	1	86.08		82.49		247.2		268.2		212.4	
		2			83.45		235.4		247.5		229.9	
2	Check	3	86.46		81.53		277.2		288.7		212.1	
		4		86.27	82.49	82.49	282.6	261.1	280.7	271.3	210.9	216.3
3	CaSO_4	5	84.53		80.57		292.9		309.7		246.5	
		6			82.49		283.2		291.9		236.6	
4	CaSO_4	7	83.76		83.45		292.3		309.7		211.2	
		8		84.14	83.93	82.64	281.4	287.5	294.6	301.5	238.3	233.1
5	$\text{CaH}_4(\text{PO}_4)_2$	9	88.01		83.05		292.3		300.1		213.0	
		10			83.45		284.1		295.9		237.9	
6	$\text{CaH}_4(\text{PO}_4)_2$	11	89.17		83.93		278.9		276.9		234.6	
		12		88.59	83.45	83.47	271.4	281.7	280.2	288.3	251.5	234.2
7	Acid Phos.	13	91.87		85.84		306.5		301.2		249.3	
		14			83.45		284.4		289.6		210.3	
8	Acid Phos.	15	93.03		80.09		282.9		279.7		222.9	
		16		92.45	80.09	82.36	277.2	287.8	282.8	288.3	234.3	228.9
9	Rock Phos.	17	87.62		83.05		285.1		291.9		212.4	
		18			85.84		305.6		275.6		203.7	
10	Rock Phos.	19	86.85		82.01		269.0		280.7		211.4	
		20		87.38		83.22	283.4	285.8	276.9	281.3	214.3	210.4
11	R'k Ph. + CaSO_4	21	87.23		83.93		274.5		300.2		226.6	
		22			85.84		272.6		283.5		220.5	
12	R'k Ph. + CaSO_4	23	87.23		85.36		235.9		293.8		217.9	
		24		87.23	87.28	85.60	230.6	257.9	253.4	282.7	210.9	218.9

and second samplings, the incubation period being 3 and 5 days respectively, but the results were not satisfactory, and the remaining tests were made by the dried-blood method. All of the results secured are given in Table VIII.

The duplicates were much more satisfactory where the casein was used, but the effects of the treatments were not clearly pronounced; the difference in ammonifying power of the soil were too small in many cases to be conclusive. The dried-blood results were more definite, but the same difficulty which is usually met with was encountered with them, that is, the impossibility of securing entirely satisfactory duplicate determinations. However, the results given in the table show certain tendencies among the treatments and it will be worth while to call attention briefly to some points which appear more or less definitely.

The calcium sulfate had the greatest effect of any of the materials on ammonification, the mono-calcium phosphate and acid phosphate were about equal in their effect, but lower than that of the calcium sulfate or the raw rock phosphate, and rock phosphate with calcium sulfate had little influence. Practically all the differences between these averages are as great as between duplicates.

The stimulative action of all these materials on ammonification is indicated by the results secured, and there are some relations evident between the ammonification results and the sulfofication tests. Thus in both cases the calcium sulfate exerted the greatest stimulative action of any of the materials used. In the sulfofication results, however, the rock phosphate alone gave practically as large an effect as the gypsum, while in ammonification it had less influence.

Again, in the sulfofication tests the acid phosphate had less effect than either the calcium sulfate or mono-calcium phosphate, while in ammonification it showed less influence than the calcium sulfate, but practically the same as the mono-calcium phosphate. In both cases the mixture of rock phosphate and calcium sulfate gave small influence.

It is impossible to explain these divergences in results, some of which owing to the difficulties encountered in the methods are not as pronounced as they should be, and indeed it is doubtful if the present results should be regarded as conclusively showing any definite differences in effect on ammonification among the various substances used. Not a large enough number of determinations were made and the duplicate results were not in sufficiently satisfactory agreement.

The stimulative action of all the substances on ammonification was, however, indicated, just as was the case with sulfofication, and hence there must be some relationship between the two processes. Of course, the same groups of organisms are not involved in the different processes, but they may belong in the same class because of their requirements for growth, especially their food materials and the most favorable mechanical soil conditions.

The differences noted in the effect of phosphorus fertilizers may have been due to different effects of phosphorus as a food material on the two

groups of bacteria, but as has been pointed out these variations were not distinctive and it is probable that the food material requirements of the different groups are not very dissimilar.

THE CROP YIELDS.

The oats were harvested just prior to maturity, and the green and dry weights secured. The crop was analyzed for nitrogen and the removal of nitrogen from the soil in the crop was calculated. All these results are given in Table IX.

TABLE IX.
THE CROP YIELDS.

Pot No.	Treatment	Green Weights Gm.	Average	Dry Weight Gm.	Average	% N. in Crop	Gp. N. in Crop	Average
1	Check	266.00		52.90		2.572	1.3605	
2	Check	262.40	264.2	49.70	51.30	2.423	1.1942	1.2773
3	CaSO ₄	263.55		50.45		2.310	1.1654	
4	CaSO ₄	243.90	253.7	49.00	49.72	2.346	1.1495	1.1574
5	CaH ₄ (PO ₄) ₂	239.60		48.00		2.677	1.2849	
6	CaH ₄ (PO ₄) ₂	271.35	255.4	50.45	49.22	2.201	1.1104	1.1976
7	Acid Phosphate	327.80		62.70		2.699	1.6923	
8	Acid Phosphate	319.90	323.9	63.00	62.85	2.561	1.6134	1.6528
9	Raw Rock Phosphate	300.00		55.00		2.751	1.5121	
10	Raw Rock Phosphate	323.70	311.9	56.50	55.75	2.652	1.4984	1.5052
11	Raw Rock Phos. plus CaSO ₄ ..	305.30		54.90		2.959	1.6244	
12	Raw Rock Phos. plus CaSO ₄ ..	277.40	291.3	51.70	53.30	2.553	1.3200	1.4722

On examining the table it is found that all the applications of phosphorous except the mono-calcium phosphate increased the crop yield. The acid phosphate gave the largest increase, much larger than that given by the raw rock. When the gypsum was applied with the rock phosphate, slightly lower yields were secured than when the rock phosphate alone was used. The difference, however, was slight and should not be considered as indicating any depression from the use of the gypsum.

The gypsum alone and the mono-calcium phosphate gave no effects. The actual average yields were slightly less than that of the check soils, but the differences in the duplicate pots were as great as those between the checks and the treated soils, and hence the results should merely indicate an absence of effect for the treatments.

It will be recalled that the soil used in this work was very low in phosphorus and hence a beneficial effect of the phosphorus fertilizers on crop yields might have been expected. It is evident from these results that when a soil is as low in phosphorus as this one was, applications of phosphorus fertilizers would prove of value. These results also indicate a superior value for the acid phosphate over the rock phosphate. No conclusions applicable to field conditions should be drawn from this single experiment, especially as it was conducted under greenhouse conditions.

The results may merely serve to indicate what *may* occur under field conditions *on this particular soil type*. No attempt has been made, therefore, to calculate the relative cost of applications and the value of the increases, which would be necessary in field tests, in order to arrive at some conclusions regarding the relative values of the applications.

Why the mono-calcium phosphate should not have brought about any increase in yield is not apparent from the results. A slight depression in the crop yield was actually observed, but it was not large enough to be distinctive, as the differences in the duplicate pots were wider than the differences between the check and treated pots, as has been noted. It appears merely, therefore, that the plants were unable to utilize the phosphorus from this compound as well as from acid phosphate. The sulfate present in the acid phosphate could not account for the greater effect of the latter material as the sulfate alone produced no effect on the crop. Possibly the acidity of the mono-calcium phosphate may explain the results, especially as this would have more effect in the absence of the calcium sulfate than where the two occur together in the acid phosphate.

The use of calcium sulfate on this soil was definitely shown to be of no value. This is as might be expected from the fact that the soil was so abnormally high in sulfur. There was evidently an abundance of sulfur present and in the presence of sufficient organic matter and lime, the process of sulfofication proceeded rapidly enough to keep the oats supplied with sulfates. Even in the presence of phosphorus, where a larger growth was secured, the sulfate had no additional effect, showing the absence of any need for sulfates on this soil.

On comparing the results of the sulfofication tests and ammonification tests with the crop yields, it is found that there were some agreements and some discrepancies in the effects of the various treatments. The gypsum exerted the greatest effect on sulfofication and likewise on ammonification, but had no influence on the crop grown. Mono-calcium phosphate likewise gave a considerable increase in sulfofying power and in ammonifying power, but had no effect on the yield of oats. Acid phosphate however increased sulfofication, ammonification and crop yield, the latter to the greatest extent of any material used, and the two former processes to as great an extent as the other substances applied. Rock phosphate increased the crop yield and the sulfofying power of the soil, but had no pronounced effect on ammonification. It is apparent, therefore, that the sulfofying power of a soil may be increased without a corresponding increase in crop yield occurring. As has been mentioned, conclusions should hardly be drawn from the ammonification results, but it seems that other factors might be of greater importance from the crop standpoint than from the standpoint of the transformation of soil nitrogen at least, in greenhouse soils.

In general, these crop yield results show that on this soil, rich in sulfur but poor in phosphorus, phosphate fertilizers produced a pronounced effect, while sulfates had no influence. The supply of sulfur and of nitrogen available for plant growth was evidently sufficient and phosphorus was the limiting factor of growth. Hence the influence of applications of materials merely increasing the supply of nitrates and sulfates was not apparent above the effect of the use of phosphorus.

CONCLUSIONS.

This experiment leads, therefore, to the following conclusions:

1. The sulfate content of the soil varied only slightly from one sampling to the next. There were no sudden or striking changes in the amount of sulfates present in the soil, kept fallow in the greenhouse.

2. The sulfate content of soils in the field is subject to the same influences as the nitrate content, but the effects are probably much less pronounced.

3. Calcium sulfate, mono-calcium phosphate, acid phosphate, rock phosphate and rock phosphate plus gypsum increased the sulfofying power of the soil. The sulfate alone and phosphates alone had greater effects than combinations of the two materials as in acid phosphate.

4. All the materials used increased the ammonifying power of the soil, but the differences between the effects of the various substances were not pronounced. The rock phosphate had less effect, however, than the other materials.

5. The sulfofication tests and ammonification tests did not always run parallel, although very similar effects of the materials used, on the two processes were noted.

6. The phosphorus fertilizers, except mono-calcium phosphate, increased the yield of oats, the acid phosphate to a greater extent than the rock phosphate. The sulfate had no effect on the crop yield. Such results were expected on this soil rich in sulfur but deficient in phosphorus. The lack of effect from the mono-calcium phosphate was probably due to the acidity, which was of more effect in the absence of the sulfate than when the two were together as in the acid phosphate.

7. The crop yields, sulfofication and ammonification results were not always parallel. In general it appeared that on this soil increases in sulfofication were not necessarily parallel with increases in yields. The ammonification results were not conclusive but indicate that materials supplying plant food constituents which are lacking in the soil may be of double value because of increases in the production of other plant food constituents in an available form.

II.

SERIES I.

THE PROPER INCUBATION PERIOD FOR TESTS OF SULFOFICATION.

In previous tests of soils for their sulfofying power by the use of free sulfur, which was found to be the best material to use, the incubation period was 10 days. It seemed desirable to ascertain whether this period of incubation allowed the greatest differentiation between soils from different sources and under varying treatments. Shorter periods of incubation were eliminated, as less satisfactory in earlier experiments and hence the tests here were carried out at 7, 10, 12 and 14 day periods.

Five soils, very different as to texture and composition, and thus presumably varying widely in sulfofying power, were selected. Fresh soil was used, being weighed out in 100-gm. quantities in tumblers, 100 mg. of sulfur added to each, and the moisture content of each of the soils adjusted to the optimum for that particular soil. The sulfates produced at the end of the various incubation periods were determined in the usual way.

TABLE X.
SULFATES PRESENT AFTER DIFFERENT PERIODS OF INCUBATION.

No.	Soils	S. as SO_4 after 7 da.	Average	S. as SO_4 after 10 da.	Average	S. as SO_4 after 12 da.	Average	S. as SO_4 after 14 da.	Average
1	Heavy black	5.72		11.76		7.92		21.47	
	Woodland soil	5.12	5.42	6.72	9.24	11.00	9.46	20.54	21.00
2	Typical sand	8.20		9.00		9.12		14.00	
	River-bank in sod	6.68	7.44	8.56	8.78	9.52	9.32	14.40	14.20
3	Humus Plot 107	5.84		5.00		4.88		6.10	
	Check	8.40	7.12	4.00	4.50	5.16	5.02	6.23	6.16
4	Humus Plot 101	9.88		10.80		18.33		30.80	
	Continuous timothy....	10.28	10.08	11.44	11.12	18.67	18.50	30.60	30.70
5	Corn-field soil	9.40		6.04		7.20		10.00	
	River terrace	8.08	8.74	6.55	6.30	8.56	7.88	10.20	10.10

On examining the results given in Table X, it is apparent that considerably larger amounts of sulfates were produced from the sulfur added, with the longer incubation periods. None of the soils showed more than a trace of sulfates at the beginning of the experiment, so the entire amount found at the end of the incubation period may be considered as produced from the sulfur added.

At the end of the 7-day period, the differences between the various soils were much too small in several cases to be conclusive. After 10 days' incubation, the amounts of sulfates produced were somewhat larger and the ranking of the soils in sulfofying power had changed materially. The duplicate determinations also agreed much better. In 12 days, the differences in sulfofying power had become still more pronounced, but the ranking of the soils was the same.

Again after 14 days' incubation, the variations among the soils were larger, but the ranking of the soils was the same as after the 10 and 12-day periods.

These results indicate, therefore, that when soils are tested for their sulfofying power by the free-sulfur-fresh-soil method, the tests should be incubated for at least 10 days to secure the proper ranking of the soils, and much better results may be secured by incubating the samples for 12 or even 14 days.

The greatest differences between various soils may be obtained by incubating for the longer period, that is, for 14 days.

SERIES II.

THE EFFECT OF GYPSUM ON SULFOFICATION.

In the earlier experiments already referred to, gypsum was found to exert a stimulative effect on sulfofication, but the amounts used were rather small and further tests seemed desirable to ascertain whether large applications would show a greater effect or whether they would depress the activities of the sulfofying bacteria. This series was planned to test this point. The soil used was a Marshall silt loam from Lee County, Iowa. It was air-dried, sieved through a twenty-mesh sieve and weighed out as usual. Sulfur in the usual amount and the special quantities of calcium sulfate were then added and thoroughly stirred in. Ten cubic centimeters of a soil infusion, made by shaking 100 gm. of fresh soil in 200 c.c. of water for five minutes were added and sufficient sterile water supplied to bring the moisture content up to the optimum. The tests were then incubated for 10 days, after which the sulfates were estimated as usual.

Table XI gives the arrangement of the experiment, together with the results secured. On examining these results, it appears that the smallest amount of gypsum had practically no effect on the sulfofying power of the soil, while the larger amounts depressed the production of sulfates. The greatest depression occurred with the use of 0.30 gm. of the sulfate, and when 0.50 gm. was added the depression was less but it was still greater than that with the 0.10 gm. of the sulfate.

The previous experiments which have shown the stimulating effect of gypsum were carried out in greenhouse soils and much smaller amounts were used than was the case here, so that these results are not in any way opposed to the earlier ones. It was apparent in those results that applications of gypsum at a rate sometimes employed in field soils stimulated sulfofication and hence it is evident that the application of gypsum cannot be increased to any appreciable extent without bringing about a depression in sulfofying power.

There could be no practical value, therefore, in making heavy applications of gypsum from the standpoint of bringing about an increase in sulfofication. Of course, these results should not be accepted as conclusive for field practice because of the fact brought out in earlier work that gypsum is rather readily assimilated in the soil, hence there was probably some assimilation in these experiments and the results secured for the treated soils may have been too small. It was impossible to ascertain the extent of the assimilation and in making the calculations, the total amount of sulfate added was subtracted from the final figure.

TABLE XI.
THE EFFECT OF CaSO_4 ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Mg. S. as SO_4 added in CaSO_4	Mg.S.as SO_4 from free S.	Av. Per Cent S. Sulfified
1	Nothing	21.0	0	21.00	
2	Nothing	21.6	0	21.60	21.30
3	0.05 gm. CaSO_4	28.4	9.35	19.05	
4	0.05 gm. CaSO_4	30.6	9.35	21.25	20.15
5	0.075 gm. CaSO_4	33.0	14.02	18.98	
6	0.075 gm. CaSO_4	32.0	14.02	17.98	18.48
7	0.10 gm. CaSO_4	37.6	18.69	18.91	
8	0.10 gm CaSO_4	36.4	18.69	17.71	18.31
9	0.30 gm. CaSO_4	62.8	56.07	6.73	
10	0.30 gm. CaSO_4	60.0	56.07	3.93	5.33
11	0.50 gm. CaSO_4	106.0	93.45	12.55	
12	0.50 gm. CaSO_4	110.0	93.45	16.55	14.55

It is safe to conclude, however, that the applications of gypsum which will give the most practically economic effect are those commonly employed in field practice.

SERIES III.

THE EFFECT OF CALCIUM CARBONATE ON SULFOFICATION.

If sulfofication is an important process occurring in field soils as seems to be the case, the effect of applications of calcium carbonate on its occurrence must be considered. Is it increased as are ammonification and nitrification, or it is decreased when the acidity of a soil is remedied by the use of limestone? This test was planned to throw some light on this point.

The same soil used in the preceding test was employed here. The soil was weighed out, the calcium carbonate in special amounts, and the sulfur added and stirred in, 10 c.c. of a fresh soil infusion applied, the moisture content adjusted to the optimum and the tests incubated for 10 days. The results of the sulfate determinations appear in Table XII. It appears clearly in this table that the use of calcium carbonate on an acid soil increased sulfofication. There was a considerable increase when the acid-

ity of the soil, the lime requirement of which was 1072 pounds per acre of 2,000,000 pounds of surface soil, was neutralized and with further additions of calcium carbonate still greater gains in sulfofication were found. The greatest gain, however, occurred with the use of 0.3 gm. per 100 gm. of soil, corresponding to 6000 pounds to the acre, and beyond that point the increases were somewhat less.

TABLE XII.
THE EFFECT OF CaCO_3 ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	21.0	
2	Nothing	21.9	21.4
3	Neutralized	27.2	
4	Neutralized	25.6	26.4
5	Neutralized plus 0.1 gm. CaCO_3	32.2	
6	Neutralized plus 0.1 gm. CaCO_3	32.8	32.5
7	Neutralized plus 0.3 gm. CaCO_3	40.6	
8	Neutralized plus 0.3 gm. CaCO_3	36.4	38.5
9	Neutralized plus 0.5 gm. CaCO_3	32.6	
10	Neutralized plus 0.5 gm. CaCO_3	33.4	33.0
11	Neutralized plus 1.0 gm. CaCO_3	35.0	
12	Neutralized plus 1.0 gm. CaCO_3	33.4	34.2
13	Neutralized plus 5.0 gm. CaCO_3	32.6	
14	Neutralized plus 5.0 gm. CaCO_3	30.4	31.5

If the applications of the carbonate had been increased still further, it is possible that the sulfofication would have decreased below that of the soil with its acidity just neutralized or even below that of the acid soil, but the amounts used here were not sufficiently large to bring about such a decrease. Inasmuch as the applications made in the field never exceed the amounts used here, there need be no apprehension of decreasing sulfofication by the use of ordinary amounts of calcium carbonate to remedy acid conditions in the soil.

On the other hand, it is evident from these results that calcium carbonate up to 6000 pounds per acre increased the sulfofying power of this soil. Larger amounts of the carbonate such as are rarely used in practice gave considerable increase in sulfofication, but these were somewhat less than those secured with the 3-ton amount.

SERIES IV.

THE EFFECT OF MAGNESIUM CARBONATE ON SULFOFICATION.

Having ascertained that calcium carbonate exerted a beneficial effect on sulfofication, it was deemed desirable to determine whether magnesium carbonate would have the same effect or not. This experiment was planned to throw some light on the question.

The soil used was the same as in the previous series. The arrangement of the test was the same as in the previous case except that no acid soil

was incubated and that magnesium carbonate was added in place of calcium carbonate. The check soils in this case were neutralized with calcium carbonate and all the other soils received additional amounts of magnesium carbonate.

On turning to Table XIII which gives the results of the tests, it is found that the smallest amount of magnesium carbonate increased slightly the sulfofying power of the soil, but the larger amounts gave gradually increasing depressions up to the largest amount employed here. It is evident that applications of magnesium carbonate in amounts greater than 2000 pounds per acre depressed the sulfofying power of this soil below that shown by the sample receiving no magnesium carbonate at all.

TABLE XIII.
THE EFFECT OF MgCO_3 ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	24.4	
2	Nothing	25.7	25.0
3	0.1 gm. MgCO_3	28.0	
4	0.1 gm. MgCO_3	31.4	29.7
5	0.3 gm. MgCO_3	26.5	
6	0.3 gm. MgCO_3	21.4	23.9
7	0.5 gm. MgCO_3	19.6	
8	0.5 gm. MgCO_3	17.1	18.3
9	1.0 gm. MgCO_3	17.0	
10	1.0 gm. MgCO_3	16.5	16.7
11	5.0 gm. MgCO_3	15.8	
12	5.0 gm. MgCO_3	15.1	15.4

Soil neutralized with CaCO_3 .

Comparing these results with those secured with the calcium carbonate in the previous test, it is found that the use of magnesium carbonate at the rate of 2000 pounds per acre gave less effect on sulfofication than the use of the same amount of calcium carbonate, both additions being made to a neutralized soil.

While, however, the use of 3 tons of calcium carbonate per acre above that necessary to neutralize the acidity of the soil, increased the sulfofying power of the soil, the application of that amount of magnesium carbonate depressed sulfofication considerably.

It is apparent, therefore, that the application of magnesium carbonate to neutral soils should be made with care, and amounts greater than 2 tons per acre might depress the sulfofying power of the soil.

Evidently the sulfofying bacteria are much less sensitive to the presence of an abundance of calcium carbonate than to the presence of much magnesium carbonate. This is in accord with other bacteriological results dealing with the transformation of soil nitrogen, and it is in accord also with many crop results.

SERIES V.

THE EFFECT OF CALCIUM AND MAGNESIUM CARBONATES ON SULFOFICATION.

This test was planned to determine the effect of calcium and magnesium carbonates combined on sulfofication. The same soil and the same arrangement of the experiment was used here as in the two previous tests, except that both calcium and magnesium carbonates were applied. The amounts of these materials combined were the same as the amounts of the single substances used in the earlier series.

The results of the tests appear in Table XIV, and an examination of this table shows that the use of calcium and magnesium carbonates in amounts larger than 2000 pounds per acre of both together depressed the sulfofying power of this soil below that of the neutral soil. The check soils here represented the soil with its entire acidity neutralized with calcium carbonate. Increasing the application of calcium and magnesium carbonates together beyond 6000 pounds per acre decreased the sulfofying power of this soil, the depression increasing with increasing amounts applied.

TABLE XIV.

THE EFFECT OF CaCO_3 PLUS MgCO_3 ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	27.0	
2	Nothing	24.8	25.9
3	0.05 gm. CaCO_3 plus 0.05 gm. MgCO_3	28.0	
4	0.05 gm. CaCO_3 plus 0.05 gm. MgCO_3	20.0	29.0
5	0.15 gm. CaCO_3 plus 0.15 gm. MgCO_3	25.7	
6	0.15 gm. CaCO_3 plus 0.15 gm. MgCO_3	24.1	24.9
7	0.25 gm. CaCO_3 plus 0.25 gm. MgCO_3	18.2	
8	0.25 gm. CaCO_3 plus 0.25 gm. MgCO_3	21.4	19.8
9	0.50 gm. CaCO_3 plus 0.50 gm. MgCO_3	16.1	
10	0.50 gm. CaCO_3 plus 0.50 gm. MgCO_3	16.4	16.2
11	2.50 gm. CaCO_3 plus 2.50 gm. MgCO_3	19.8	
12	2.50 gm. CaCO_3 plus 2.50 gm. MgCO_3	22.3	21.0

It is apparent, therefore, that on this soil applications of calcium carbonate produced greater effects on sulfofication than the use of magnesium carbonate or combinations of the two carbonates. It is further evident the use of magnesium or dolomitic limestones on this soil, after its acidity has been neutralized with calcium carbonate may lead to a depression in sulfofying power, if the amounts used exceed 2000 pounds to the acre. On the other hand, non-magnesian limestones up to 6000 pounds per acre increased the sulfofying power of the soil, and in larger applications, produced smaller effects on sulfofication, but no actual depressions.

CONCLUSIONS.

These tests lead to the following conclusions:

1. In the use of the free-sulfur-fresh-soil method for testing the sulfofying power of soils, the incubation period should be 14 days at room temperature to give the most conclusive results. Ten day's incubation gave the relative sulfofying powers of soils quite accurately, but the differences were much more distinctive for the longer period.

2. Calcium sulfate in ordinary applications had no detrimental effect on sulfofication, but very large applications might decrease the rate of oxidation of sulfur.

3. Calcium carbonate in ordinary applications on acid soils, increased sulfofication considerably and even in excessive amounts affected sulfur oxidation favorably.

4. Magnesium carbonate in small amounts increased sulfofication, but in large amounts depressed it even below that in the same soil with its acidity unneutralized.

5. Magnesium carbonate and calcium carbonate in combination exerted a beneficial influence on sulfofication when used in small amounts. Larger applications, however, depressed the oxidation of sulfur. The effects of the combined material were less than those of the calcium carbonate alone.

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