AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date Thesis presented May 2, 1940

Title Effect of Waste Sulfite Liquor on Soil Properties and Plant Growth

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Sunflowers and corn were grown in Newberg loam soil treated with varying amounts of waste sulfite liquor. It was found that waste sulfite liquor equivalent to 84 tons per acre could be added before toxicity was noticeable. The addition of nitrogen as nitrate with waste sulfite liquor stimulated plant growth.

Waste sulfite liquor additions to the soil gave an immediate increase in the water soluable potassium, sulfate, and calcium content. Upon incubation the amount of potassium and sulfates increased, but the calcium content decreased. The addition of waste sulfite liquor to the soil caused an immediate decrease in hitrate nitrogen, which decreased further upon incubation. The addition of urea decreased further upon incubation. The addition of urea with waste sulfite liquor to the soil gave nitrate production upon incubation. The amount of nitrate produced was governed by the carbon nitrogen ratio of the added urea and waste sulfite liquor.

Waste sulfite liquor, when added to the soil, increased the carbon dioxide evolution except with additions equivalent

to 560 tons per acre. The addition of wheat straw with waste sulfite liquor gave an increase in carbon dioxide evolution. Calcium nitrate when added alone to the soil, had a depressing effect on the carbon dioxide evolution. When calcium nitrate was added to the soil with waste sulfite liquor or wheat straw the carbon dioxide evolution increased. There was a general increase in microbial population with waste sulfite liquor additions either alone or with calcium nitrate or wheat straw, except in the case of the larger application of waste sulfite liquor.

The pH of the soil was decreased by waste sulfite liquor additions, which gradually increased upon incubation.

EFFECT OF WASTE SULFITE LIQUOR ON SOIL PROPERTIES AND PLANT GROWTH

by

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A THESIS

submitted to the

OREGON STATE COLLEGE

in partial fulfillment of
the requirement for the
degree of

DOCTOR OF PHILOSOPHY

June 1940

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ACKNOWLEDGEMENT

I wish to take this opportunity to thank Dr. R. E. Stephenson, Dr. W. E. Caldwell and Dr. W. B. Bollen for their guidance and help. Their inspirational directions and criticisms were invaluable in the preparation and completion of this thesis.

CONTENTS

EFFECT OF WASTE SULFITE LIQUOR ON SOIL PROPERTIES AND PLANT GROWTH

INTRODUCTION

In the production of wood pulp for paper or rayon manufacture, wood chips are digested with sulfite leach liquor. The leachate, which contains large amounts of lignin, sulfur, and some sugars, as well as various other substances, is termed "waste sulfite liquor." Disposal of this waste is a serious problem. Various investigations directed toward possible utilization of this material have met with only moderate success.

Phillips et al. (7) point out that the annual discharge from wood pulp mills in this country contains about 1,500,000 tons of lignin, which is equivalent to some 27,000,000 tons of liquor. This material carries a large quantity of combined sulfur, calcium, and potassium, and other substances in varying quantities. The approximate specific gravity of the liquor is 1.12, and the loss from drying is about 89 per cent (6). As may be seen from table 1, waste sulfite liquor contains considerable material that should affect soil fertility and the growth of plants.

Though these data are subject to large variation and some error, they serve to give an idea of the quantity of waste liquor and its contents yearly dumped into streams, in many instances causing damage as well as loss of materials that might be recovered and utilized.

TABLE 1

APPROXIMATE COMPOSITION OF WASTE SULFITE LIQUOR*

	Per cent	Tons
Total solids	10.7	2,889,000
Sulfur	0.98	264,600
Calcium	0.48	129,600
Potassium	0.031	5,370
Nitrogen	0.004	1,155
Lignin	5.53	1,500,000
Phosphorus	0.0017	459
Sugars	1.81	488,700
Carbon	4.15	1,120,500

* Potassium, phosphorus, calcium and carbon were determined by the author on a sample of liquor from the Rayonier pulp mill. Nitrogen was obtained from data by Phillips et al. (7). Other data are from Partansky and Benson (6).

The bulkiness of the waste product probably prohibits at least at the present time, the use of the material in the raw state as a fertilizer. There is considerable interest, however, in knowing what the effect is when the liquor, in the raw state, is added to the soil. The relatively high sulfur content of the liquor lends interest to the question of using the material on sulfur-deficient soils. Likewise, the high acidity suggests possibilities for correcting alkali soils. There is also the possibility that the lignin of the liquor might contribute to humus formation in soils.

Use of the raw liquor on soils has been commonly regarded as undesirable because of the toxic effect on plants and microorganisms, although no experimental work to support this idea has been reported. Investigations to determine the specific effects of raw waste sulfite liquor on plant growth, solubility of soil nutrients, and microbial processes in the soil were carried out on Newberg loam, a slightly acid soil of high natural fertility. This thesis reports the results of this study.

METHODS

To determine toxic concentrations, soil was treated with varying amounts of liquor in lacquered cans in the greenhouse. The cans held one pound of soil. Sunflowers and corn were grown as indicator plants. The sunflower seeds were allowed to germinate and sprout through the soil before the liquor was applied. In treating with liquor, 2 cc. were put on the soil after seed germination, and the treatment was doubled or tripled weekly until the desired amount of liquor had been added. After a 6-week period of growth, the plants were measured for height and then harvested for dry weights. In the case of corn, the liquor was all applied at the time of planting. After an 8-week period of growth, the plants were measured for height and harvested. Treatments were in quadruplicate, and five plants were grown in each can, giving 20 plants for each treatment.

The effect of the liquor on soluble nutrients was determined by treating the soil in the same way and incubating for varying periods without planting. Water extracts (1:5) were used for analysis. Potassium was determined by the cobaltinitrite method (5). The calcium was precipitated as the oxalate and titrated with standard permanganate in the usual way. The sulfate sulfur was precipitated and weighed as barium sulfate. Nitrates were determined by the phenoldisulfonic acid method (2).

The effect of the liquor on microological processes was studied by treating 1-kgm. portions of soil in a respiration apparatus and aerating with CO_2-free air. The carbon dioxide produced by the organisms bringing about decomposition was determined by the method of Bollen and Ahi (3); the carbon dioxide was absorbed in sodium hydroxide and double titrated with standard sulfuric acid, phenolphthalein and brom phenol blue being used as indicators.

In the microbial counts, peptone-glucose-acid agar was used for molds, and sodium albuminate agar as described by Fred and Waksman (4) was used for bacteria and actinomyces. Duplicate plates from appropriate dilutions were poured and the numbers of colonies counted after incubation of 28°C. until the colonies could be distinguished.

The titration curves were made from data obtained by using 10-gm. samples of soil and adding acid, alkali, or liquor as indicated with enough water to make a 1:5 suspension. The pH was measured with a glass electrode after 30 minutes of shaking. Ten grams soil and ten cubic centimeters liquor were mixed together and titrated with alkali in a similar manner.

RESULTS AND DISCUSSION

1. Effect of w.s.l.* on Plant Growth

The data in table 2 show that sunflowers grew satisfactorily in Newberg loam with concentrations of w.s.l. as high as 22 cc. which is the equivalent of 61.6 tons an acre of 2,000,000 pounds of soil. There was no toxic effect from additions up to 30 cc., which corresponds to 84 tons of liquor per acre. The growth response is shown in the column "index of growth." This figure is calculated by taking the greatest height attained by any treatment (60 cm.) as 100. Other treatments then produce heights which are a certain per cent of the maximum. One gram dry weight taken as a standard for a single plant is used in the same way for weight comparisons. The height and weight percentages obtained in this manner are added and divided by two. This figure is termed the index of growth.

Table 2 shows that the index of normal growth of the control under conditions provided was 41.1. A figure much lower than this indicates toxicity. In the 30- and 48-cc. applications the average weights of the plants are high because only five of the twenty original plants survived, yet

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* Throughout this paper, w.s.l. is used as an abbreviation for waste sulfite liquor.

the reduced heights of these plants shows the toxic effect of the liquor. Can 7, (fig. 1) received a 30 cc. application of w.s.l., and only one stunted plant grew showing definite toxicity.

TABLE 2

EFFECT OF W.S.L. ON THE GROWTH OF SUNFLOWERS

W.S.L. Treatments	Average Height of Plants	Dry Weight of Plants	Average Weight of Plants	Index of Growth
cc.	cm.	gm.	gm.	
Control	38.0	3.54	0.19	41.1
2	40.7	4.12	0.21	44.5
4	45.6	4.97	0.26	51.0
8	45.3	5.32	0.28	51.7
16	43.1	3.58	0.24	47.9
22	41.2	3.18	0.23	45.8
30	32.8	1.14	0.23	38.8
48	28.8	0.76	0.25	35.5

The effect of w.s.l. on the growth of corn is shown in table 3. In this case application of 8 cc. of w.s.l. which corresponds to 22 tons per acre proved toxic to corn. The index of growth is calculated by using 65.4 cm. as the maximum height and 2.10 gms. as maximum weight for any single plant. The height of the corn was measured by running the hand up the stalk of the plant until the tips of the top leaves were left and then measuring with a meter stick from the base of the stalk to this high point.

TABLE 3

EFFECT OF W.S.L. ON THE GROWTH OF CORN

W.S.L. Treat-ments	Average Height of Plants	Dry Weight of Plants	Average Weight of Plants	Index of Growth
cc	cm.	gm.	gm.	
Control	37.6	9.66	0.54	41.4
2	37.8	10.88	0.54	41.7
4	40.0	10.73	0.53	43.2
8	36.9	8.34	0.42	38.1
16	37.4	8.07	0.40	38.0
32	24.0	2.87	0.14	21.6
48	5.0	0.17	0.008	3.8

All the plants, including those of the control, were spindly with yellow leaves. Can 6 and 7 (fig. 2) that received 32- and 48-cc. applications of w.s.l. respectively, showed definite toxicity. This is also shown in the index of growth (table 3) which is far below that of the control. Cans 4 and 5 receiving 8- and 16 cc. of w.s.l. respectively have plants which are less sturdy than the control, but are approximately the same height as the control. The growth response is shown in the index of growth which is less than that of the control. Cans 2 and 3 receiving 2- and 4-cc. w.s.l. are taller and heavier than the control, and give accordingly higher indices of growth.

Large applications of w.s.l. are more toxic to corn than to sunflowers. This may be due to the reduction in nitrate concentration which takes place on the addition of large amounts of w.s.l. to Newberg loam soil, causing spindly plants and a definite yellowing of the leaves. Corn may have a higher nitrogen requirement than the sunflowers.

In order to overcome any nitrogen deficiency caused by additions of w.s.l. to the soil, a series of treatments was run in which nitrogen was added to the samples during the growing period. Table 4 shows the treatments that were made. The N-only was a solution containing 0.02 gm. nitrogen as nitrate per 100 cc. During the growing period 1000 cc. of this solution were applied which corresponds to 0.2 gms. nitrogen. The complete nutrient used was a solution containing 0.035 gm. potassium, 0.02 gm. nitrogen as nitrate, 0.0217 gm. phosphorus as phosphate, 0.028 gm. calcium, 0.0168 gm. magnesium and 0.0224 gm. sulfur as sulfate per 100 cc. A total of 1,000 cc. of this solution were applied during the growing season.

The fertilization with complete nutrient more than doubled the growth of corn, as shown by the index of growth (table 4). The plants were sturdy with a full rich green color. The nutrient with N-only caused a definite increase in growth over the control. There was a general increase in yield with increased amounts of w.s.l. used with nutrient N-only. The maximum growth as indicated by the index of

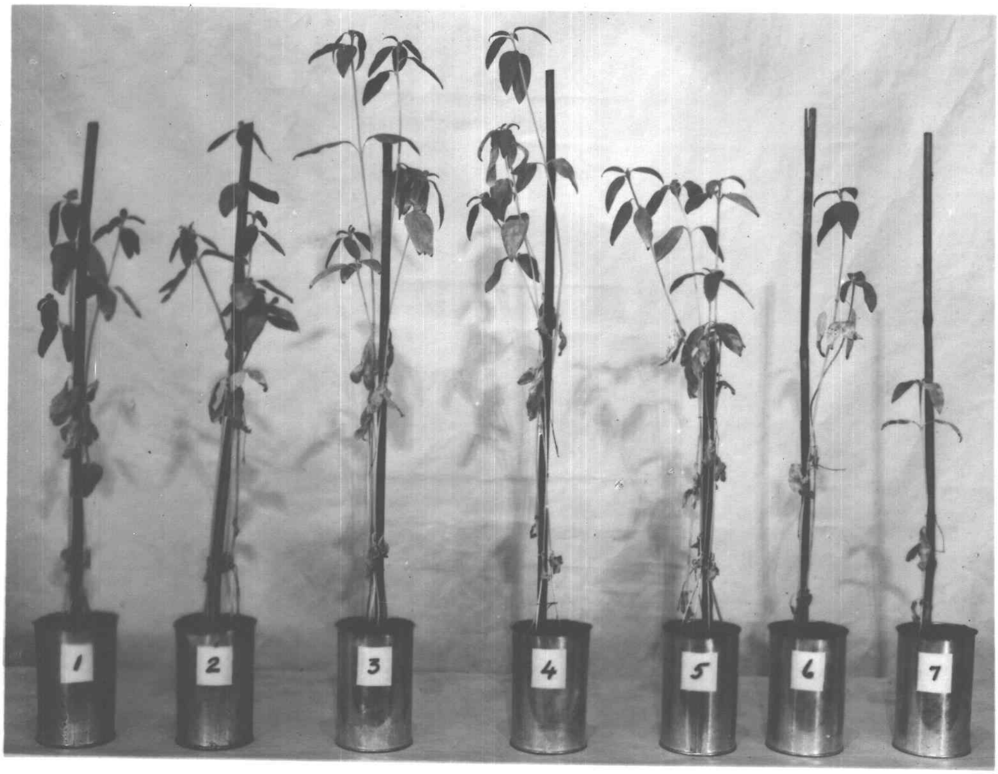


Fig. 1

1-Control; 2-2 cc. w.s.l.; 3-4 cc. w.s.l.; 4-8 cc. w.s.l.; 5-16 cc. w.s.l.; 6-22 cc. w.s.l.; 7-30 cc. w.s.l.

Fig. 2

1-Control; 2-2 cc. w.s.l.; 3-4 cc. w.s.l.; 4-8 cc. w.s.l.; 5-16 cc. w.s.l.; 6-32 cc. w.s.l.; 7-48 cc. w.s.l.

growth was with the 16 cc. w.s.l. which corresponds to 44 tons per acre. This may be seen in Fig. 3. Can 6 represents the 16 cc. w.s.l. and N-only applications. This figure also shows the increase in growth due to the increased application of w.s.l. Can 8 received complete nutrient solution and the plants were very sturdy. The addition of nitrogen with w.s.l. definitely increased the growth of corn in Newberg loam soil.

TABLE 4

EFFECT OF W.S.L. AND NITROGEN AS NITRATE ON THE GROWTH OF CORN

W.S.L. Treatments	Average Height of Plants	Dry Weight of Plants	Average Weight of Plants	Index of Growth
	cm.	gm.	gm.	
Control	37.6	9.66	0.54	41.4
Complete Nutrient	63.1	42.00	2.10	98.1
N-only	44.8	17.40	0.87	54.8
N-only, 2 cc. w.s.l.	49.5	20.82	1.04	62.5
N-only, 4 cc. w.s.l.	50.7	21.49	1.08	64.2
N-only, 8 cc. w.s.l.	55.3	26.17	1.32	73.5
N-only, 16 cc. w.s.l.	56.3	27.05	1.36	75.4
N-only, 32 cc. w.s.l.	57.6	25.63	1.28	74.4

Table 5 shows the effect of w.s.l. used as a source of sulfur. The nutrient S-omit was the same as the complete nutrient except for the omission of sulfur; 1000 cc. of this nutrient was used. This nutrient caused an increase in yield of about 50 per cent over the control. There was a general increase in growth with increased additions of w.s.l. with the S-omit nutrient. Growth was nearly as good when 32 cc. of liquor was used as a source of sulfur as when sulfur was supplied in the usual way in the complete nutrient. Fig. 4, shows the general increase in growth due to increased additions of w.s.l. with the other nutrients. This shows that the liquor was a satisfactory source of sulfur.

The effect of w.s.l. and various nutrients upon the growth of sunflowers (9) is approximately the same as in the case of corn. Definite increases in yield were obtained with the application of w.s.l. used as a source of sulfur with and without the addition of lime.

TABLE 5

EFFECT OF W.S.L. USED AS A SOURCE OF SULFUR ON THE GROWTH OF CORN

W.S.L. Treatments	Average Height of Plants	Dry Weight of Plants	Average Weight of Plants	Index of Growth
	cm.	gm.	gm.	
Control	37.6	9.66	0.54	41.4
Complete Nutrient	63.1	42.00	2.10	98.1
S-omit	47.5	21.16	1.06	61.3
S-omit, 2 cc. w.s.l.	62.2	32.23	1.69	87.6
S-omit, 4 cc. w.s.l.	60.5	32.60	1.68	86.1
S-omit, 8 cc. w.s.l.	62.5	34.44	1.72	88.5
S-omit, 16 cc. w.s.l.	60.9	34.66	1.73	87.6
S-omit, 32 cc. w.s.l.	65.4	34.76	1.74	91.4

Fig. 3

1-Control; 2-N-only; 3-2 cc. w.s.l. and N-only; 4-4 cc. w.s.l. and N-only; 5-8 cc. w.s.l. and N-only; 6-16 cc. w.s.l. and N-only; 7-32 cc. w.s.l. and N-only; 8-complete nutrient.

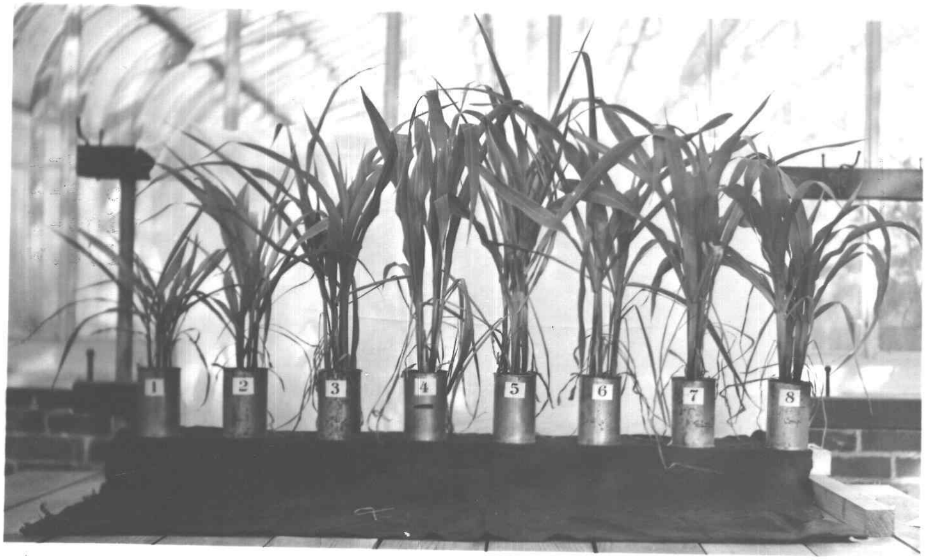


Fig. 4

1-Control; 2-S-omit; 3-2 cc. w.s.l. and S-omit; 4-4 cc. w.s.l. and S-omit; 5-8 cc. w.s.l. and S-omit; 6-16 cc. w.s.l. and S-omit; 7-32 cc. w.s.l. and S-omit; 8-complete nutrient.

2. Effect of w.s.l. on water-soluble nutrients of the soil

Newberg loam treated with w.s.l. as indicated in table 6, showed an increase in water soluble potassium, calcium, and sulfate. The nitrate content decreased immediately on the addition of the liquor. Upon incubation, there was a complete loss of nitrate. This may be due in part to the chemical reaction between the sulfites or sulfides present in the liquor and the soil nitrates. Since there was a decrease in nitrates immediately, this explanation seems reasonable. The total absence of nitrates at the end of the 2-week period may be caused in part by microbial activity. The sugars and other easily decomposable organic compounds in the liquor stimulated the microorganisms, which utilized the nitrates and other available nitrogen. The acidity caused by the w.s.l. also inhibits nitrification.

Soluble potassium was increased fivefold by the addition of 16 cc. of w.s.l. Since the potassium content of the liquor is 3.5 mgm. per cc., the liquor added accounts for most of the increase. There was a general increase of water-soluble potassium on incubation, which may be caused by the slow base-exchange reaction taking place, as well as by solvent action of carbon dioxide resulting from microbial activity.

TABLE 6

EFFECT OF W.S.L. ON WATER-SOLUBLE NUTRIENTS
OF NEWBERG LOAM SOIL

Nutrient	Incubation Period	w.s.l. treatment per 400 grams soil				
		0 cc.	2 cc.	4 cc.	8 cc.	16 cc.
	Weeks	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Calcium	0	20	28	100	150	410
	2	27	41	80	145	331
	5	35	44	75	160	262
	10	40	47	61	144	224
Sulfur as Sulfate	0	2	8	19	26	48
	2	2	20	46	50	120
	5	5	33	61	98	149
	10	3	30	72	148	212
Potassium	0	4	5	11	13	20
	2	4	5	13	13	20
	5	6	8	12	16	23
	10	6	8	11	19	25
Nitrogen as Nitrate	0	8	7	7	5	4
	2	8	0	0	0	0
	5	9	0	0	0	0
	10	9	0	0	0	0

The sulfur present in the w.s.l. is in the form of sulfides, sulfites, sulfates and organic sulfur compounds. Immediate increase of the water-soluble sulfates is due to their presence in the liquor. Incubation for two weeks doubled the sulfate concentration in almost every treatment. Longer periods of incubation increased the sulfate content, but the increase was not so pronounced as in the initial 2-week period. This sulfur oxidation may be brought about in part by the oxidation of the reduced forms of sulfur, by oxygen of the soil air, as well as by the action of sulfur oxidizing molds and bacteria. Not all of the sulfur added as w.s.l. was converted to sulfates. The maximum conversion after 10 weeks represents about 60 per cent of the total sulfur added in the liquor.

Table 6 shows an immediate increase in the amount of water-soluble calcium, due to the fact that the liquor contains 4 mgm. calcium per cc. In the heavier treatments, the amount of calcium found exceeds the water-soluble calcium content of the soil and the calcium added in the w.s.l. by a large amount (230 p.p.m. excess for the 16-cc. treatment in original analysis). The acidity of the liquor when added to the soil, caused a base replacement, freeing calcium from the colloidal complex by substitution with hydrogen. On the other hand, incubation decreased the soluble calcium in every treatment. This reversal may be explained on the basis of the solubility of calcium sulfite in water. In the formation of sulfite leach liquor, which is used in the extraction of cellulose from wood, limestone is treated with sulfurous acid. The following equations indicate the reaction:

$$CaCO_3 + H_2SO_3 \longrightarrow CaSO_3 + H_2CO_3$$

$$CaSO_3 + H_2SO_3 \longrightarrow Ca(HSO_3)_2$$

The solubility of $CaSO_3.2H_2O$ in water is but 43 p.p.m. at 18° C. (8). The $CaSO_3$ is highly soluble in sulfurous acid. In w.s.l. there is enough free sulfurous acid to keep the calcium sulfite in solution. This is the case in the immediate analysis of the treated soil. On incubation free H_2SO_3 is oxidized, and the pH of the treated soil increases (table 8). This decrease of acidity causes the formation of insoluble calcium sulfite as follows:

$$Ca(HSO_3)_2 \longrightarrow CaSO_3 + H_2SO_3$$

Plants grown in soils treated with large additions of w.s.l. become stunted and exhibit yellow leaves significant of nitrogen deficiency. Additions of nitrogen with w.s.l. produced a marked increase in yield and gave plants which were normal with a full rich green color. Direct evidence of this deficiency is shown in table 6, where successive treatments with w.s.l. caused a complete loss of soil nitrates. Table 7 gives data which shows that an excess of nitrate can be produced in the presence of w.s.l. in the soil if enough nitrogen is added to maintain a suitable C/N ratio.

The results in table 7 show that additions of w.s.l. to the soil caused disappearance of nitrate nitrogen as is the case in table 6. Nitrification in the control was rather high during the 80-day incubation period, but increased additions of w.s.l. reduced or completely stopped this process. The addition of 2 grams of calcium carbonate reduced the acidity of the soil, making it a more favorable medium for the nitrification process, and in almost every case, there was an increase in nitrates over that obtained in the presence of w.s.l. alone.

The source of nitrogen used in this experiment was urea which contains 46.7 per cent nitrogen. At the end of the 80-day incubation period all of the nitrogen in urea was not present in the nitrate form. The addition of lime along with the urea did not increase nitrification. Increased additions of w.s.l. cut down the nitrification of urea. Table 7 shows the C/N ratio of the added mixture of urea and w.s.l. The narrower the C/N ratio, the more nitrification took place, but even in the treatment with the narrowest C/N ratio where w.s.l. was present the amount of nitrates accumulated was wide, the microorganisms were activated or increased in number to such an extent that they utilized nitrates in large quantities while decomposing the easily decomposable carbon containing compounds which were in the liquor.

The addition of lime along with w.s.l. and urea, did not effect the nitrification except with the higher treatments with w.s.l. The increased nitrification resulted from neutralizing the acidity of the liquor. Soil treated with the largest amount of w.s.l. (8 cc.) in all cases had little or no nitrate at the end of the 80-day incubation period.

TABLE 7

EFFECT OF TREATMENTS ON NITRIFICATION IN NEWBERG LOAM

Treatments per 100 Grams Soil	C/N Ratio*	N as NO_3				
		Incubation period, days				
		15	30	80	15	80
		p.p.m.	p.p.m.	p.p.m.	pH	pH
Control		17.5	31.5	44.0	5.7	5.6
Urea 0.02 gm.		30.0	69.0	97.5	5.8	5.5
Urea 0.02 gm. 1 cc. w.s.l.	5.6/1	16.0	46.0	88.0	5.8	5.5
Urea 0.02 gm. 2 cc. w.s.l.	11.2/1	11.0	31.0	65.0	5.6	5.3
Urea 0.02 gm. 4 cc. w.s.l.	22.5/1	1.0	10.0	27.0	5.5	5.3
Urea 0.02 gm. 8 cc. w.s.l.	55/1	T ≁	T	T	5.3	5.3
1 cc. w.s.l.		10.0	23.0	28.0	5.5	5.7
2 cc. w.s.l.		4.5	15.0	19.5	5.3	5.5
4 cc. w.s.l.		T	6.0	12.5	5.2	5.4
8 cc. w.s.l.		T	T	T	5.1	5.3
2 gm. $CaCO_3$		22.0	49.0	54.0	7.2	7.1
2 gm. $CaCO_3$ 1 cc. w.s.l.		12.5	24.5	35.0	7.0	6.9
2 gm. $CaCO_3$ 2 cc. w.s.l.		6.5	13.0	25.0	6.8	6.7
2 gm. $CaCO_3$ 4 cc. w.s.l.		T	4.0	9.6	6.6	6.5
2 gm. $CaCO_3$ 8 cc. w.s.l.		T	2.0	3.0	6.4	6.4
0.02 gm. Urea 2 gm. $CaCO_3$		46.0	69.0	100	7.1	6.9
0.02 gm. Urea 2 gm. $CaCO_3$ 1 cc. w.s.l.		25.0	38.0	82.0	7.0	6.8
0.02 gm. Urea 2 gm. $CaCO_3$ 2 cc. w.s.l.		25.0	38.0	65.0	6.8	6.7
0.02 gm. Urea 2 gm. $CaCO_3$ 4 cc. w.s.l.		17.5	26.0	26.0	6.6	6.6
0.02 gm. Urea 2 gm. $CaCO_3$ 8 cc. w.s.l.		9.0	9.0	16.0	6.4	6.4

T ≁ = Trace (less than 0.1 p.p.m.)
*C/N = Carbon and nitrogen ratio of the urea and w.s.l. added to the soil

3. Effect of w.s.l. on the Biological Activity of the Soil

Carbon dioxide evolution from Newberg loam treated with w.s.l. and wheat straw is shown in figure 5. The increased rate of carbon dioxide evolution with time is indicated by curves in which carbon dioxide evolution is expressed in milligrams of carbon evolved. The control is taken as zero and plotted as the x-axis. Since the greater part of the carbon in the soil is present in the organic matter, the carbon dioxide evolved serves as an indication of microbial activity and organic matter decomposition. The results show that any addition of other organic substances with the sulfite liquor increased the amount of carbon evolved in the earlier period of incubation. Upon further incubation, the curves decrease in slope and finally become nearly horizontal, indicating approach to an equilibrium. In all treatments with w.s.l., there is an increase in the carbon dioxide evolved. This is due to the easily decomposed organic substances, such as sugars, contained in the liquor. In case of the 500 cc. w.s.l. treatment, there was less carbon dioxide evolved than from the control in the early periods of incubation. This may have been due to the unfavorable pH caused by such large additions, which retarded biological activity. After the toxicity was overcome, the carbon dioxide evolution increased over the control.

Addition of straw to the soil increased the carbon dioxide evolution over that of the control. Increasing additions of w.s.l. with straw, as shown in figure 5, increased the carbon dioxide evolution with the exception of the 500 cc. w.s.l. treatment, which during the first part of the incubation period gave less carbon dioxide than was evolved from the control.

The effect of treating Newberg loam with wheat straw and w.s.l. on the number of molds, bacteria and actinomyces is given in table 8. The original soil, having a moisture content of 20 per cent and a saturation capacity of 60 per cent, had 7,250,000 bacteria and actinomyces and 38,000 molds per gram of dry soil. After a ten day incubation period there was an increase in the number of molds with most of the treatments, with the exception of the heavier applications of w.s.l. The 50 cc. treatment gave irregular results. At the end of the incubation period the number of molds was large, particularly with the larger application. Molds are not inhibited by the acidity due to the w.s.l.

After the 10-day period of incubation, there was a general increase in the number of bacteria except in the case of the treatments with large amounts of w.s.l. In the case of the 100-, 250- and 500 cc. applications of w.s.l. the numbers of bacteria were greatly decreased. This was probably due to the inhibiting effect of the increase in

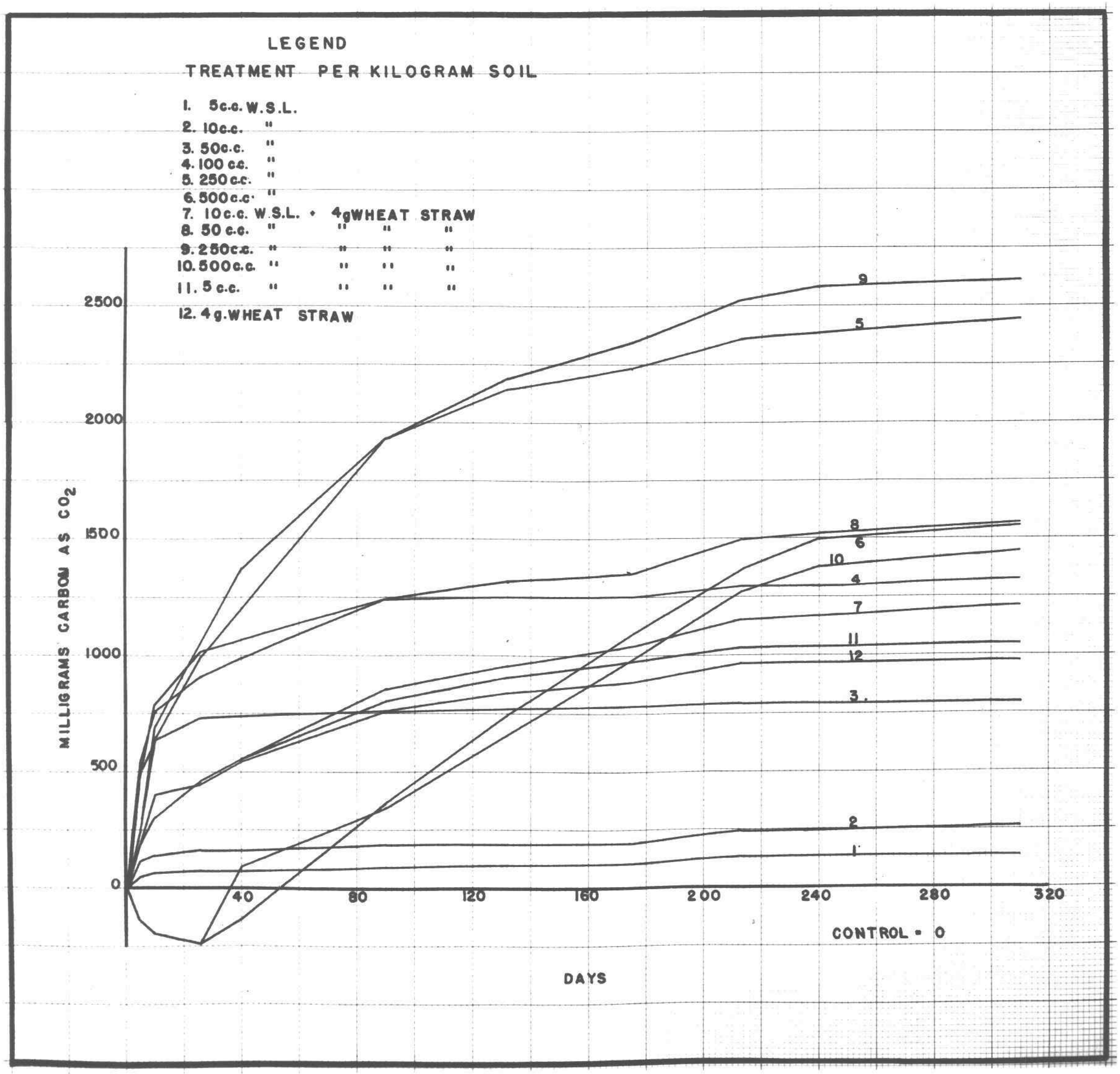
TABLE 8

EFFECT OF W.S.L. AND STRAW UPON THE MICROBIAL POPULATION OF NEWBERG LOAM SOIL. NUMBERS PER GRAM

Treatment per Kilogram Soil	Molds x 500		Bacteria x 50,000		Actinomyces x 50,000	
	Incubation period, days					
	10	310	10	310	10	310
Control	85	210	400	48	3	7
5 cc. w.s.l.	115	260	700	37	5	10
10 cc. w.s.l.	70	385	250	97	0	21
50 cc. w.s.l.	44	325	100	50	0	15
100 cc. w.s.l.	204	1400	1002	75	0	12
250 cc. w.s.l.	5	320	0.1	0	0	0
500 cc. w.s.l.	0.25	775	0.7	3	0	3
5 cc. w.s.l. 4 gm. straw*	150	420	1250	40	20	5
10 cc. w.s.l. 4 gm. straw	170	655	320	83	5	10
50 cc. w.s.l. 4 gm. straw	350	950	400	88	0	15
250 cc. w.s.l. 4 gm. straw	37	1500	0	1	0	0
500 cc. w.s.l. 4 gm. straw	0.25	310	0	0	0	0
4 gm. straw	78	450	1000	49	4	20

* Wheat straw

Fig. 5


LEGEND
TREATMENT PER KILOGRAM SOIL
1. 5c.c. W.S.L.
2. 10c.c. "
3. 50c.c. "
4. 100 c.c. "
5. 250 c.c. "
6. 500 c.c. "
7. 10c.c. W.S.L. + 4g WHEAT STRAW
8. 50 c.c. " " " "
9. 250c.c. " " " "
10. 500c.c. " " " "
11. 5 c.c. " " " "
12. 4 g. WHEAT STRAW
MILLIGRAMS CARBON AS CO_2
DAYS
CONTROL = 0

acidity caused by the addition of the liquor. Treatment with wheat straw caused an increase in the number of bacteria in the earlier days of incubation. The addition of liquor with wheat straw caused a general increase in the number of bacteria, except with the heavier w.s.l. applications. The increase in number of bacteria somewhat irregularly corresponds to the increase in the amount of carbon dioxide evolved from the soil. The largest application of w.s.l. caused a marked and permanent reduction in the plate count, but only a temporary reduction in the carbon dioxide evolution. At the end of the incubation period the number of bacteria in all cases was low; this corresponds with the leveling off of the curves in figure 5, indicating the tendency toward a microbial equilibrium.

Heavy applications of w.s.l. inhibited the development of actinomyces, which decompose the more stable forms of organic matter. After the soil had reached a somewhat stable condition, where soil processes were returning to the original normal, the number of actinomyces increased. The more simple organic compounds added had been decomposed, leaving the more resistant residues which were then decomposed slowly by the actinomyces. After 310 days incubation the 250- and 500 cc. w.s.l. treatments still inhibited the growth of actinomyces.

The results in figure 6 show the effect of calcium nitrate, w.s.l. and wheat straw on the carbon dioxide evolution of Newberg loam soil. The control is taken as zero and plotted as the x-axis. The addition of calcium nitrate to the soil decreased the evolution of carbon dioxide. This added nitrogen was immediately available for anabolic requirements of microorganisms and a reduced decomposition of organic matter resulted. With an adequate supply of easily available inorganic nitrogen the organisms were not required to decompose organic nitrogen compounds and hence less carbon dioxide was evolved. This circumstance, therefore, explains the decrease in carbon dioxide production, even though increased plate counts were obtained when calcium nitrate was added. The addition of w.s.l. carrying a large proportion of readily decomposable constituents along with calcium nitrate increased the evolution of carbon dioxide over that of the soil alone, or soil plus calcium nitrate. The effect of calcium nitrate upon w.s.l. decomposition is about the same as the effect on decomposition of native soil organic matter. This may be seen by comparing figures 5 and 6.

The addition of wheat straw alone produced an increase in all plate counts (table 8) and in carbon dioxide evolution. Calcium nitrate added with wheat straw gave an additional increase in carbon dioxide evolution and also gave an additional increase in the number of microorganisms.

The stimulating effect of the added nitrate with straw is in contrast with the depressing effect obtained when calcium nitrate was added to the soil alone, because the straw provides readily decomposable carbon compounds. This situation also obtains with combined additions of w.s.l., calcium nitrate and wheat straw. Waste sulfite liquor in the largest application proved definitely toxic. This is shown by permanent reduction in the number of bacteria and actinomyces and a temporary reduction in the number of molds and in the rate of carbon dioxide evolution. Subsequent increase in carbon dioxide evolution was apparently due to mold activity since these organisms showed a striking increase in number at the end of the experiment. A large proportion of the molds developed were tentatively identified as Penicillium luteum. This is of interest inasmuch as Abbot (1) has shown that this penicillium oxidizes sulfur. Some preliminary experiments carried out with a pure culture of this mold isolated from the w.s.l. treated soil, showed that it had definite sulfur-oxidizing power.

The number of bacteria at the end of the incubation period was smaller than at the beginning (table 9). The carbon dioxide evolution in all cases approached a common minimum rate as shown in both figures 5 and 6, by the leveling off of the curves. The stabilization of the numbers of bacteria and actinomyces in almost every treatment and the common minimal carbon dioxide evolution toward the end of the incubation period, indicate a virtual microbial equilibrium.

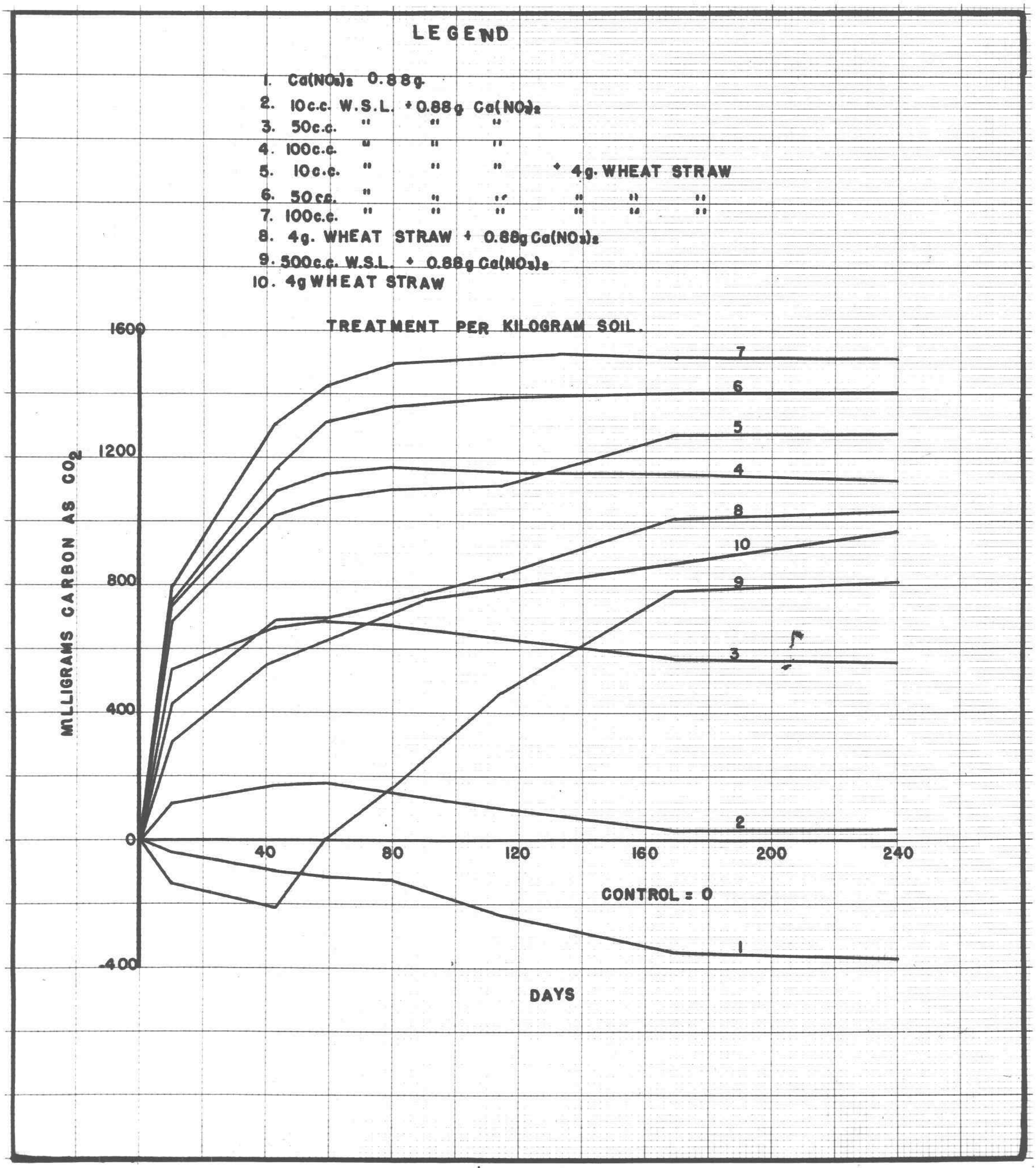
TABLE 9

EFFECT OF WHEAT STRAW, CALCIUM NITRATE AND W.S.L. ON MICROBIAL POPULATION OF NEWBERG LOAM. NUMBERS PER GRAM

Treatment per Kilogram Soil	Molds x 500		Bacteria x 50,000		Actinomyces x 50,000	
	Incubation period, days					
	10	240	10	240	10	240
Control	120	340	175	55	30	12
0.88 gm. $Ca(NO_3)_2$	75	440	800	61	76	10
10 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$	120	800	115	55	18	9
50 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$	100	1080	300	36	18	10
100 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$	125	1240	300	31	12	11
10 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$ 4 gm. wheat straw	95	850	155	155	11	18
50 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$ 4 gm. wheat straw	150	1500	3	55	0*	13
100 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$ 4 gm. wheat straw	160	2150	75	99	2	18
0.88 gm. $Ca(NO_3)_2$ 4 gm. wheat straw	125	750	240	190	20	30
500 cc. w.s.l. 0.88 gm. $Ca(NO_3)_2$	2	3500	0	0*	0	0*

* No colonies on plates of 1/5000 dilution

Fig. 6


LEGEND
1. Ca(NO3)2 0.88g.
2. 10c.c. W.S.L. + 0.88g Ca(NO3)2
3. 50c.c. " " "
4. 100c.c. " " "
5. 10c.c. " " " + 4g. WHEAT STRAW
6. 50cc. " " " " " "
7. 100c.c. " " " " " "
8. 4g. WHEAT STRAW + 0.88g Ca(NO3)2
9. 500c.c. W.S.L. + 0.88g Ca(NO3)2
10. 4g WHEAT STRAW
TREATMENT PER KILOGRAM SOIL.
MILLIGRAMS CARBON AS CO2
1600
1200
800
400
0
-400
40
80
120
160
200
240
7
6
5
4
8
10
9
3
2
1
CONTROL = 0
DAYS

4. Effect of w.s.l. on Soil Reaction

The w.s.l. used in these experiments had a pH of 1.0. When soil is treated with a solution of such high acidity, the soil reaction is quickly changed. The titration curves are given in figure 7. Newberg loam titrated with 0.1 N sulfuric acid maintains a lower pH than when titrated with w.s.l. When soil treated with 10 cc. w.s.l. is titrated with 0.093 N sodium hydroxide, 2.5 cc. of sodium hydroxide must be added to reach pH 7, which corresponds to 0.0091 gm. sodium hydroxide or 0.012 gm. calcium carbonate.

TABLE 10

EFFECT OF W.S.L. ON THE pH OF NEWBERG LOAM

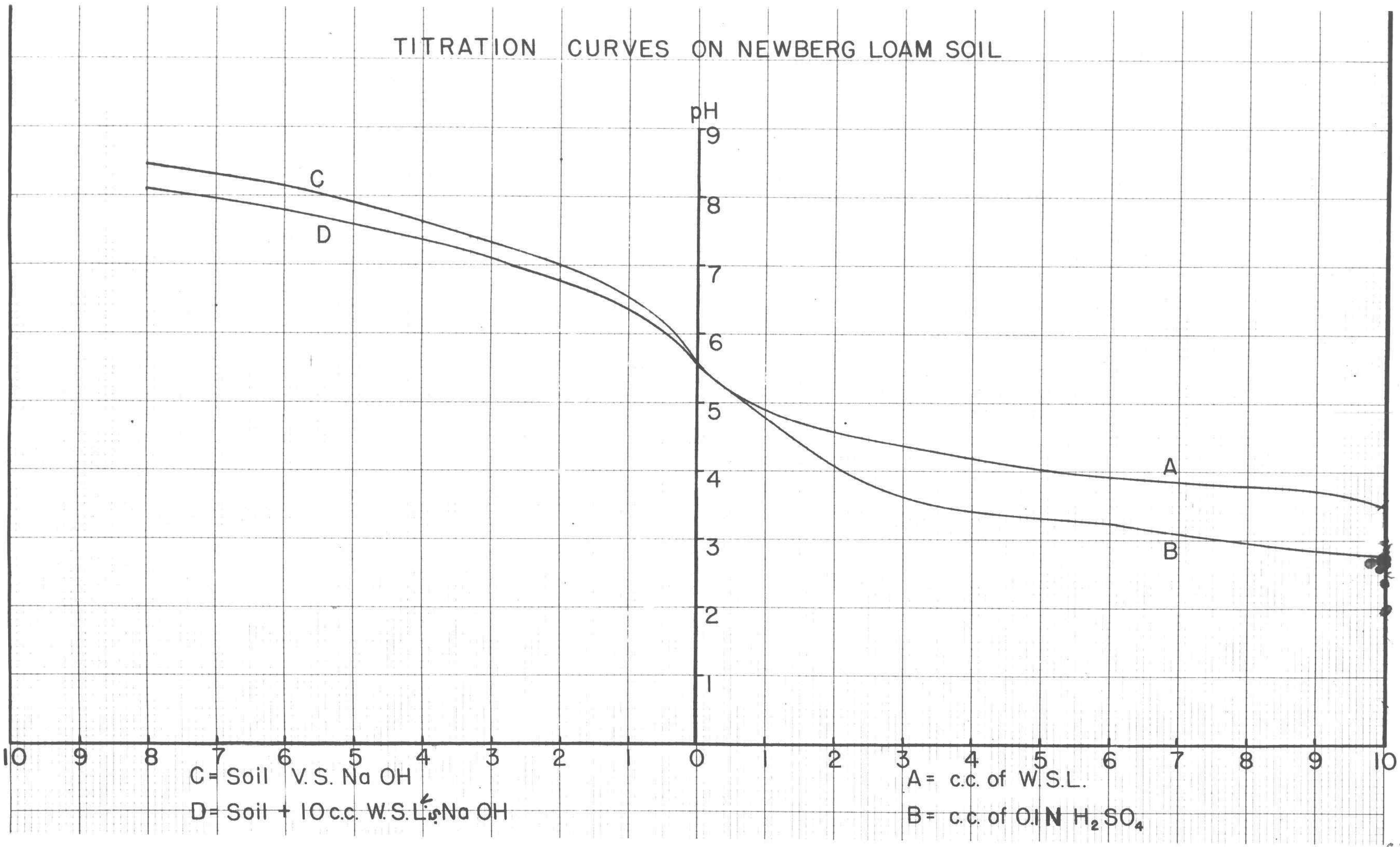
w.s.l. Treatment per 400 gm. soil	0 Weeks	2 Weeks	5 Weeks	10 Weeks
cc.	pH	pH	pH	pH
0	5.7	5.8	5.8	5.8
2	5.3	5.4	5.7	5.8
4	5.1	5.25	5.5	5.6
8	4.9	5.1	5.25	5.4
16	4.7	4.9	5.1	5.3

Table 10 gives the effect of w.s.l. on the pH of Newberg loam when incubated at room temperature. The pH of the soil drops with increased additions of w.s.l. The return to normal pH is rapid with the lighter applications. With the 8- and 16-cc. treatments the pH is almost back to normal after 10 weeks. The increase in pH is probably due to the base exchange reaction of the sulfurous acid with the colloidal matter of the soil, resulting in slightly soluble and slightly ionized acids. This effect is seen in table 6, when because of base exchange, the amount of water-soluble calcium is increased by w.s.l. additions.

The data in table 7 shows the effect of additions of urea and calcium carbonate with w.s.l. on the soil reaction. Addition of 0.02 gm. urea to the soil increased the pH of the soil in the early periods of incubation. This is due to the ammonification of urea. After the 80-day incubation period the acidity had increased because of the oxidation of the ammonia into nitrates. The addition of w.s.l. with urea gave a general decrease in pH with increased additions of w.s.l. The acidity of the w.s.l. causes this decrease in pH. The nitrification of the urea causes a further increase in acidity.

The addition of calcium carbonate to the soil increases the pH, which decreases a little on longer incubation. The application of urea with calcium carbonate shows little difference in pH over that of the calcium carbonate treatment in the 15-day incubation period. A longer period of incubation increases acidity due to the nitrification of urea causing a subsequent decrease in pH. Increased w.s.l. applications with urea and calcium carbonate gave a general decrease in pH, due to the acidity of the liquor, but showed very little difference from the treatments with w.s.l. and calcium carbonate alone.

Fig. 7


TITRATION CURVES ON NEWBERG LOAM SOIL
pH
9
8
7
6
5
4
3
2
1
C
D
A
B
10 9 8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10
C= Soil V.S. Na OH
D= Soil + 10 c.c. W.S.L. vs Na OH
A= c.c. of W.S.L.
B= c.c. of 0.1 N H₂SO₄

SUMMARY

Waste sulfite liquor was added to Newberg loam soil in amounts ranging from the equivalent of 5.6 to 134.4 tons per acre per 2,000,000 pounds of soil. The effect on plant growth, water soluble nutrients, microbial activity and population, and soil reaction was determined.

Waste sulfite liquor equivalent to 84 tons per acre could be added to the soil without becoming toxic to the growth of sunflowers. A larger quantity of the liquor produced a definite stunting of growth with few plants surviving. Additions of liquor equivalent to 22 tons per acre increased the growth of sunflowers. In the case of corn, the results were similar with respect to toxicity. The addition of liquor alone was not beneficial to corn, which may be due to the large nitrate requirement for growth of corn. The application of w.s.l. at either the time of seeding or after germination of the seeds, produced similar results.

The addition of nitrate nitrogen with w.s.l. stimulated the growth of corn. The maximum benefit from the use of nitrate resulted when the treatment was equivalent to 44 tons of w.s.l. per acre. The addition of nitrate provided nitrogen to overcome the effect of the large amount of energy material supplied by the w.s.l.; the supply of available

nitrogen must meet the needs of the active bacteria in decomposition as well as the needs of the growing crop. Waste sulfite liquor was a satisfactory source of sulfur when other nutrients were supplied.

The w.s.l. supplied water soluble calcium, potassium and sulfur compounds. The water soluble calcium of the soil increased with additions of the liquor to a much greater extent than the equivalent of the calcium supplied by the liquor. The acidity of the liquor caused a base exchange in which calcium from the colloidal matter of the soil was replaced by the hydrogen from the acids in w.s.l. In the heavier treatments with w.s.l. the amount of water soluble calcium decreased with incubation indicating a possible oxidation of the free sulfurous acid, leaving the slightly soluble calcium sulfite.

The water soluble potassium increased immediately with the addition of the liquor due to the small amount of soluble potassium present in the liquor. There was a small general increase in water soluble potassium upon incubation which may be due to slow base exchange or to the potassium liberated as a result of organic matter decomposition.

The water soluble sulfates increased due to the sulfates present in the liquor. There was a marked increase in sulfates on incubation, which was caused by the oxidation of the sulfur compounds in the liquor by soil air, bacteria and sulfur-oxidizing molds.

The addition of w.s.l. to the soil caused an immediate decrease in nitrate nitrogen, which decreased further upon incubation. The spindly growth of the plants bearing yellow leaves indicated a nitrogen deficiency. The neutralization of w.s.l. with calcium carbonate had little effect on the disappearance of nitrates except in the higher w.s.l. treatment. When urea, an organic nitrogen compound, was added to the soil with w.s.l. nitrate production was governed by the carbon-nitrogen ratio of the added mixture. A narrow C/N ratio gave the most nitrate production. Again, the addition of calcium carbonate with w.s.l. and urea produced no effect over that of w.s.l. and urea alone, except in the higher w.s.l. treatments.

When soil was treated with w.s.l., the evolution of carbon dioxide was increased, except in the larger applications, due to the addition of easily decomposed organic compounds present in the liquor. The increased carbon dioxide evolution was associated with the general increase in microbial population. In the case of the largest w.s.l. treatment which was equivalent to 560 tons per acre, the amount of carbon dioxide evolved was far below that of the control, indicating a definite toxicity. After a longer period of incubation, the toxic effects were overcome and the carbon dioxide evolution surpassed that of the control.

The microbial population was very small in the early period of incubation, but had increased above that of the control at the end of the incubation period. The addition of wheat straw with w.s.l. increased carbon dioxide evolution over that of w.s.l. alone. These treatments also increased the microbial population, which corresponds with the increased carbon dioxide evolution.

The carbon dioxide evolution of the soil was decreased by treatment with calcium nitrate. In this case the number of microorganisms increased. Since the added calcium nitrate supplied immediately available nitrogen for microbial use, there was consequently less organic matter decomposition. The addition of w.s.l. or wheat straw with calcium nitrate gave an increase in the amount of carbon dioxide evolved. In these cases, enough easily decomposable carbon compounds were added to balance the added nitrogen, and increased decomposition took place. The increase in carbon dioxide evolution corresponds generally with increased plate counts.

The pH of the soil decreased with increased additions of w.s.l. This was due to the acidity of the liquor. On incubation the pH slowly returned to the normal. In case of the smaller treatments the increase in pH was more rapid than with larger applications of liquor. This increase in pH was probably due to the slow base exchange reaction in which the hydrogen of the acids in w.s.l. replaced cations from the colloidal complex, forming an insoluble acid which was slightly ionized, thereby giving a higher pH.

CONCLUSION

1. Waste sulfite liquor in concentrations below 84 tons per acre was not toxic to sunflowers grown in Newberg loam soil. It proved a satisfactory source of sulfur for corn grown on sulfur deficient soils, definite increases in yield being obtained.

2. Waste sulfite liquor increased the concentration of potassium, calcium and sulfate salts in the water extract of the soil, and decreased the nitrate content. Additions of urea with w.s.l. resulted in nitrate formation.

3. Waste sulfite liquor increased the rate of carbon dioxide evolution and the microbial population in the soil, when added alone, or in the presence of wheat straw, or with calcium nitrate. Concentrations of w.s.l. equivalent to 560 tons per acre decreased the rate of carbon dioxide evolution until the toxicity was overcome. The microbial population was inhibited by such concentrations. Calcium nitrate decreased the rate of carbon dioxide evolution and increased the microbial population.

4. Waste sulfite liquor additions to Newberg loam soil lowered the pH of the soil suspension; on incubation the pH gradually increased.

BIBLIOGRAPHIES

1. Abbott, E.V. The occurance and action of fungi in soils. Soil Sci. 16: 207-216, 1923

2. Association of Official Agricultural Chemists. Official and tentative methods of analysis, ed. 4 Washington, D.C., 1919

3. Bollen, W.B., and Ahi, S.M. Effect of alkali salts on general microbial function in soil. Soil Sci. 46: 287-305, 1938

4. Fred, E.B., and Waksman, S.A. A tentative outline of the plate method for determining the numbers of microorganisms in the soil. Soil Sci. 14: 27-28, 1922

5. Hibbard, P.L., and Stout, P.R. Estimation of potassium by titration of the cobaltinitrite with potassium permanganate. Jour. Assoc. Off. Agr. Chem. 16: 137-140, 1933

6. Partansky, A.M., and Benson, H.K. Methods of analysis of sulphite waste liquor. Paper Trade Jour. 102: 29-35, 1936

7. Phillips, M., Goss, M.J., Brown, B.E., and Reid, F.R. The ammoniation of waste sulfite liquor and its possible utilization as a fertilizer material. Jour. Agr. Res. 53: 209, 1936

8. Seidell, A. Solubilities of Inorganic and Organic Compounds, ed. 2. D. Van Nostrand Co. New York, 1919

9. Spulnik, J.B., Stephenson, R.E., Caldwell, W.E., and Bollen, W.B. Effect of waste sulfite liquor on soil properties and plant growth. Soil Sci. 49: 37-47, 1940

SUPPLEMENTAL BIBLIOGRAPHIES

The following list of references deal with various works which has been done on waste sulfite liquor. The material presented in these references is not closely related to the subject matter of the thesis and was not, therefore, included in the original bibliographies.

1. Harnist, C. The utilization of sulfite waste liquor with consideration of recent results of lignin investigations. Chem. Ztg. 56: 529-31, 1932

2. Kobe, K.A., Layman, J.H. and Ambruster, F.R. Ammoniation of sulfite waste liquor. Ind. Eng. Chem. 28: 571-2, 1936

3. Kobe, K.A. Utilization and disposal of sulfite waste liquor. Sewage Works Jour. 9: 1019-23, 1937

4. Levine, M., Nelson, G.H., Anderson, D.Q. and Jacobs, R.B. Wastes I. Lignin and microbial decomposition. Ind. Eng. Chem. 27: 195-200, 1935

5. McGeorge, W.T. Base-exchange property of organic matter in soils. Arizona Agri. Expt. Sta. Tech. Bulletin 30, 1930

6. McGeorge, W.T. Organic compounds associated with base exchange reactions in soils. Arizona Agri. Expt. Sta. Tech. Bulletin 31, 1931

7. Phillips, M. The Chemistry of Lignin, IV. Lignin from oat hulls. Jour. Amer. Chem. Soc. 52: 793-797, 1930

8. Pinck, L.A., Howard, L.B. and Hilbert, G.E. Nitrogenous composition of ammoniated peat and related products. Ind. Eng. Chem. 27: 440-5, 1935

9. Schwalbe, C.G. The manufacture of sulfite wood pulp and its waste liquor. Chem. Ztg. 54: 4-5, 1930

10. Smith, F.B., Brown, P.E. The decomposition of lignin and other organic constituents by certain soil fungi. Jour. Amer. Soc. Agron. 27: 109-19, 1935

11. Waksman, S.A. and Iyer, K.R. Contribution to our knowledge of the chemical nature and origin of humus. III. The base exchange capacity of synthesized humus, (ligno-protein) and of natural humus complexes. Soil Sci. 36: 57-67, 1933

12. Wetz, H. Sulfite waste liquor and possibilities of using it. Farben-Ztg. 44: 659-60, 1939

Patents

Bornemann, F. and Uhl, Karl. German patent 660,622 (cl 16.11) Chemical Abstracts, 32: 6866, 1938

Farhenind, I.G. German patent 599,801 (cl 16.14) Chemical Abstracts 28: 7413, 1934

Formhals, R. United States patent 2,117,087 Chemical Abstracts 32: 5150, 1938

Franz, A. and Palm, A. German patent 561,487 Chemical Abstracts 24: 1081, 1933

Paulson, P.A. German patent, 660,507. Chemical Abstracts 32: 6866, 1938