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TEXAS AGRICULTURAL EXPERIMENT STATION

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DIVISION OF CHEMISTRY

OCCURRENCE OF NITRITES IN SOILS

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†As of July 1, 1930.

The production of assimilable nitrogen from organic nitrogenous matter in the soil is an important factor in soil fertility, which has been extensively studied, but the presence of appreciable amounts of nitrites under some conditions has hitherto been overlooked.

Nitrites may occur in large quantity in cultures used in nitrification experiments, contrary to the general opinion that their amounts are generally small and relatively unimportant. This discovery opens a large field of work to ascertain the extent of occurrence, conditions of occurrence, and the scientific and practical importance of the production and occurrence of nitrites in soils. The possibility that nitrites may be present should be considered in all nitrification work.

Nitrites may occur in soil cultures alone and in those which have received ammonium sulphate or other nitrogenous additions. They were found in such important types as Norfolk fine sand and Lake Charles clay loam. Soils which have a low capacity for producing nitrates may form large amounts of nitrites. Nitrites may persist in the soil or in soil extracts for several weeks. Magnesium carbonate and calcium carbonate may favor the formation of nitrites. Water equivalent to 50 per cent of the capacity of the soil was more favorable to production of nitrites than smaller or larger amounts. The work on nitrites is being actively continued.

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OCCURRENCE OF NITRITES IN SOILS

G. S. FRAPS AND A. J. STERGES

Nitrogen, one of the most important elements of plant food, occurs in the soil chiefly as insoluble organic compounds. These compounds cannot be used directly as food for plants, but must first be changed to soluble organic compounds, to ammonia, or to nitrates. While ammonia can be assimilated by plants (4), it is generally believed that nitrates are used more extensively than ammonia by cultivated plants in ordinary arable soils.

On account of its importance, the process of nitrification has received extensive study both in the field and in the laboratory (1, 7, 8, 9, 10, 11, 12). The capacity of various soils to produce nitrates, the production of ammonia and nitrates from various fertilizing materials, the effect of temperature, moisture, and other conditions upon the rapidity of nitrification, and the quantity of nitrates in field soils under various conditions, are some of the topics which have been studied. It has been shown (5) that the nitrates produced in soils are related to the nitrogen taken by crops in pot experiments, just as the total nitrogen of the soil (3, 6) is also so related. Nitrification is known to be caused by bacterial action, the nitrogen in the organic matter being changed first to ammonium compounds, to nitrites, and then to nitrates (4), thus being made available for use by plants.

It has been generally assumed that nitrites are extremely transitory, and that they occur only in amounts that are practically negligible; consequently the amounts of nitrites found have not been determined in nitrification experiments.

In a comprehensive study of nitrification at the Texas Experiment Station, irregularities were observed which rendered necessary a comparison of several methods for estimating nitrates.

On account of discrepancies between the amounts of nitrates as determined by the colorimetric method (phenol-disulphonic acid) and the reduction method (zinc ferrous sulphate), a test was made for nitrites in certain cultures, and they were found in large quantity. As much as 98 parts per million of nitrous nitrogen was found in cultures of soils which had received no nitrogenous additions, and as much as 226 parts per million in cultures which received ammonium sulphate. Further studies, which are here described, showed the presence of nitrites in considerable amounts.

Method of work

The method used for the nitrification experiments was similar to the so-called tumbler method. For the usual procedure, to 200 grams of soil was added 0.1 gram of nitrogen in a solution of ammonium sulphate, together with 10 c.c. of inoculating liquid made from Lufkin fine sandy loam and sufficient water to equal 50 per cent of the water capacity of the soil. The inoculating liquid was made by shaking 100 grams of field soil with 200 c.c. of water and allowing the soil to settle. The supernatant liquid was used. The weighed beakers were left in a moist incubator at 35° C. for four weeks, the water lost being replaced two times a week by adding sufficient water to the surface of the culture to restore the loss in weight. At the end of the period, nitrate nitrogen was estimated by the phenol-disulphonic acid method and nitrites by the diphenylamin method. The procedure was varied for the different kinds of experiments.

Nitrites in soils without additions of nitrogenous materials

While the quantity of nitrous nitrogen in cultures of soils which received no nitrogenous addition was frequently small, yet it sometimes reached comparatively large amounts (Table 1). All these cultures had received carbonate of lime, and were incubated 4 weeks in the usual way. The amount of nitrite nitrogen varied from 6 to 98 parts per million. The nitrites exceeded the nitrates in two cases. The percentage of nitrous nitrogen in the combined nitric and nitrous nitrogen varied from 11 to 74 per cent.

Nitrification capacity as measured by nitric nitrogen alone, and by nitric and nitrous nitrogen combined

The relative nitrifying capacity of a number of soils for ammonium sulphate was compared with a standard soil, Houston black clay (29423), and some results from two sets are given in Table 2. All these soils except the standard originally had a low nitrifying power for ammonium sulphate. Calcium carbonate (1 per cent) was added to all except the standard, to see if such addition would improve the nitrifying capacity. Blank cultures (not shown) without the addition of ammonium sulphate were made for each soil, and these blanks have been deducted from the total with the results shown in the table.

High nitrate production occurred in nearly all the soils here reported. When the nitrate production was low, the nitrite production might be high. When the nitrate production was high, the nitrite production was usually low. The production of nitrate nitrogen was low in most of these soils, even though they received calcium carbonate. When the nitric and nitrous nitrogen together were considered, the relative power to oxidize nitrogen was much higher.

The exact significance of this fact remains to be seen, but it is no doubt of importance. As pointed out previously by the senior author (2), where there are wide differences in the nitrifying capacity of soils for organic matter, the differences are much narrower if the pro-

Table 1—Nitric and nitrous nitrogen in soils without nitrogenous addition

	Soil type, county and depth	In parts per million of soil			In percentage of total N.	
		Nitric nitrogen	Nitrous nitrogen	Total	Nitric	Nitrous
5939	Lake Charles clay, Harris county, 0″–12″	65	22	87	75	25
5958	Moderately good black land, Comanche county, 9″–21″	96	98	194	49	51
20720	Lake Charles clay loam, Harris county, 0″–7″	78	13	91	86	14
20721	Lake Charles clay loam, Harris county, 7″–19″	35	15	50	70	30
20727	Lake Charles clay loam, Harris county, 7″–19″	11	31	42	26	74
20729	Lake Charles clay, Harris county, 7″–19″	56	12	68	82	18
21068	Trinity clay, Rockwall county, 7″–19″	49	6	55	89	11
22227	Vernon very fine sandy loam, Wichita county, 7″–19″	36	6	42	86	14

Table 2—Relative capacity of soils treated with calcium carbonate to oxidize sulphate of ammonia

	Soil type, county and depth	Nitric nitrogen parts per million	Nitrous nitrogen parts per million	Total nitrous and nitric parts per million	Rank based on nitric nitrogen per cent	Rank based on nitrous and nitric nitrogen per cent
29423	Houston black clay, Bell county, Standard	356	....	356	100	100
5935	Lake Charles clay, Harris county, 0″–12″	13	70	83	4	25
5958	Moderately good black land, Comanche county, 9″–21″	0	42	42	0	13
12594	Norfolk fine sand, Camp county, 0″–6″	0	70	70	0	21
12648	Miller fine sandy loam, Brazos county, 10″–20″	102	53	155	30	46
29423	Upland black land, Bell county, Standard	273	0	273	100	100
20720	Lake Charles clay loam, Harris county, 0″–7″	0	226	226	0	83
20721	Lake Charles clay loam, Harris county, 7″–19″	0	193	193	0	71
20726	Lake Charles clay loam, Harris county, 0″–7″	20	191	201	7	74
20727	Lake Charles clay loam, Harris county, 7″–19″	10	155	165	4	60
20729	Lake Charles clay, Harris county, 7″–19″	0	133	133	0	45
21068	Trinity clay, Rockwall county, 7″–19″	176	91	267	67	97
21070	Houston black clay, Rockwall county, 7″-19″	57	140	197	22	72
22227	No. 6 Vernon fine sandy loam, Wichita county, 7″–19″	0	74	74	0	27

Table 3—Nitrous and nitric nitrogen after different periods of time, in parts per million

Time	Lake Charles clay soil 5935, 0″–12″		Norfolk fine sand, 12594, 0″–6″		Norfolk fine sandy loam subsoil 12648		Lake Charles clay loam 20720, 0″-7″	
	Nitrous	Nitric	Nitrous	Nitric	Nitrous	Nitric	Nitrous	Nitric
After 7 days	0	0	0	0	3	1	24	5
After 14 days	3	6	55	14	122	23	273	47
After 21 days	280	24	208	15	250	21	360	40
After 28 days	510	58	192	11	290	46	384	50
After 35 days	500	42	188	11	300	58	295	135
After 64 days	450	117	164	24	93	275	5	540

Table 4—Effect of moisture content on production of nitrous and nitric nitrogen, parts per million

	Soil 5935		Soil 12594		Soil 12648		Soil 20720	
	Nitrous	Nitric	Nitrous	Nitric	Nitrous	Nitric	Nitrous	Nitric
Water added								
25% of the water capacity	20	4	47	8	59	7	112	18
35% of the water capacity	59	18	144	17	140	23	250	60
50% of the water capacity	255	44	216	18	260	82	310	140
80% of the water capacity	96	11	4	0	4	15	176	24
50% of the water capacity with stirring	510	58	192	20	290	46	384	50

duction of both nitrates and ammonia combined is considered. A similar condition appears to occur, to some extent, with respect to nitrates and nitrites. Soils which have a low power to produce nitrates may have a much higher power to produce nitrites.

Effect of time of incubation

Table 3 shows the quantities of nitrite and nitrate nitrogen found in cultures incubated for different periods of time. The production of nitrites was small the first week, a little more the second; then it proceeded rapidly and reached a maximum in 28 days, after which it decreased. The production of nitrates was much slower than the production of nitrites in these soils.

Effect of different amounts of water

Water was added in various percentages of the water capacity of the soil and the cultures incubated for four weeks as described. The maximum production of both nitrites and nitrates occurred with 50 per cent of the water capacity (Table 4). Previous studies have shown this to be the favorable proportion for nitrates, and is the amount used ordinarily in our work. The percentage of water most favorable for nitrite production seemed also to be most favorable for nitrite production.

The cultures designated "50 per cent water capacity with stirring" had been used in the test for the effect of time and a portion had been removed each week, after stirring. The results were irregular, some being nearly the same as those not stirred, while one showed a wide difference, which is, however, in accord with other work (see Table 3).

Effect of calcium and magnesium carbonates

The effect of calcium carbonate and of magnesium carbonate on the oxidation of ammonia, is shown in Table 5. The addition of 1 per cent of calcium carbonate to the cultures containing ammonium sulphate, caused a decided increase in the production of both nitrous and nitric nitrogen. Addition of magnesium carbonate increased nitrite and nitrate production as compared with ammonium sulphate alone, but compared with calcium carbonate nitrite production was less in two of the four soils, and nitrate production was less in three of the four soils. The production of nitrite nitrogen was affected by calcium or magnesium carbonate in a manner similar to their effect on nitrate production.

Effect of varying amounts of ammonium sulphate

Increasing the addition of sulphate of ammonia to 1000 parts per million of nitrogen did not affect nitrite or nitrate production in some soils, but decreased both in soil 12594, nitric nitrogen in soil 12648,

Table 5—Effect of various additions on the production of nitrous and nitric nitrogen, parts per million

	Soil 5935		Soil 12594		Soil 12648		Soil 20720	
	Nitrous	Nitric	Nitrous	Nitric	Nitrous	Nitric	Nitrous	Nitric
No addition	0	8	0	51	0	56	4	140
Ammonium sulphate (500 parts nitrogen per million)	3	13	0	0	0	90	0	80
Ammonium sulphate 500 N. p. m. and calcium carbonate	255	44	218	18	260	82	310	140
Ammonium sulphate 1000 N. p.m. and calcium carbonate	290	42	15	10	212	24	392	48
Ammonium sulphate 250 N. p. m. and calcium carbonate	90	43	11	164	64	176	88	172
Ammonium sulphate 500 N. p. m. and magnesium carbonate	280	41	4	0	168	16	300	29
Calcium carbonate 1%	54	30	59	4	5	66	5	88
Magnesium carbonate 1%	58	28	0	20	4	58	6	78

Table 6—Effect of standing upon nitrous nitrogen in soil, parts per million

		In soil		In solution			
		When set aside after 4 weeks incubation	After 6 weeks in room temperature	After 24 hours	After 48 hours	After 7 days	After 30 days
20727	Lake Charles clay loam, Harris, 7″–19″	186	150	156	160	156	152
20729	Lake Charles clay, Harris, 7″–19″	155	250	250	245	245	250
20729	Lake Charles clay, Harris, 7″–19″	190	360	368	352	368	360
21068	Trinity clay, Rockwall, 7″–19″	97	18	29	19	19	20
21068	Trinity clay, Rockwall, 7″–19″	103	29	30	31	29	31
21069	Houston black clay, Rockwall, 0″–7″	81	40	40	40	41	42
21070	Houston black clay, Rockwall, 7″–19″	142	68	72	68	69	70
21070	Houston black clay, Rockwall, 7″–19″	96	30	30	30	31	30
22227	Vernon very fine sandy loam, Wichita, 7″–19″	79	96	105	98	104	105
22227	Vernon very fine sandy loam, Wichita, 7″–19″	117	143	145	143	147	144

and nitrous nitrogen in soil 20720 (Table 5). Decreasing the sulphate of ammonia to 250 parts per million of nitrogen decreased the production of nitrites, but increased that of nitrates with three of the four soils.

The failure of ammonium sulphate to nitrify in some soils has been ascribed (4) to an injurious action of the ammonia on the bacteria. The decrease caused by the smaller amount of the ammonium sulphate and the increase by the larger amounts seems to be in favor of this theory so far as nitrates are concerned.

Persistence of nitrites in cultures

Some of the cultures were set aside at room temperature for six weeks. The nitrites before and after standing are given in Table 6. In some cases there were increases, in others decreases, but the striking point is that the nitrites were not quickly converted into nitrates, but persisted for several weeks in the soil in large quantity.

Persistence of nitrites in solution

The extracts from the cultures which had stood for six weeks were set aside, and nitrites determined after 24 and 48 hours. The results immediately after extraction, in the second column of Table 6, can be compared with those after standing. It was thought that nitrites were so unstable that inaccurate results would be obtained if the solutions were not tested immediately, but these solutions are evidently stable, since all the results are in the limit of error. Even after several weeks, the quantity of nitrites had hardly changed.

Nitrites in field soils

To what extent nitrites occur in field soils remains to be ascertained. Nitrites were estimated in 12 samples of field soils (April, 1929) but only .04 to .06 parts per million of nitrous nitrogen was found. It is possible that nitrites occur in much larger amounts, especially on certain soils in the presence of organic or commercial fertilizers, or under some conditions of moisture or temperature.

Nitrites in laboratory samples

Nitrites were found in small amounts in 24 samples of dry soils on hand in the laboratory. The amounts found are not significant, ranging from .02 to .05 parts per million.

Nitrites and nitrates from urea

The nitrates and nitrites formed from urea were compared with those formed from ammonium sulphate in a standard soil. The results are given in Table 7. A sample of each soil with no addition was included,

Table 7—Nitric and nitrous nitrogen from urea, in parts per million

		Nitrogenous additions	Nitric N	Nitrous N
29423	Upland black land, Bell county	ammonium sulphate	408.0	11
29423	Upland black land, Bell county	urea	344.0	0
29423	Upland black land, Bell county	nothing	74.0	0
5935	Lake Charles clay, (probably), 0″–12″, Harris county	urea	25.0	48
5935	Lake Charles clay, (probably), 0″–12″, Harris county	nothing	2.5	0
5937	Lufkin fine sandy loam, 0″–7″, Brazos county	urea	36.0	100
5937	Lufkin fine sandy loam, 0″–7″, Brazos county	nothing	100.0	0
5958	Moderately good black land, 9″–21″, Comanche county	urea	35 0	220
5958	Moderately good black land, 9″–21″, Comanche county	nothing	51.3	106
6010	Lufkin fine sand, (probably), 0″–12″, Angelina county	urea	80.0	33
6010	Lufkin fine sand, (probably), 0″–12″, Angelina county	nothing	52.5	0
6268	Upland sandy loam, 0″–12″, Colorado county	urea	17.5	15
6268	Upland sandy loam, 0″–12″, Colorado county	nothing	33.0	0
7169	Norfolk fine sandy loam, 0″–6″, Upshur county	urea	47.5	5
7169	Norfolk fine sandy loam, 0″–6″, Upshur county	nothing	40.0	0

so that the change caused by the addition could be ascertained. Much more nitrate was produced from the ammonium sulphate than from the urea in the standard soil. Nitrites were produced from the urea in many of the soils, less often from the soil itself. The urea caused a reduction in the nitric nitrogen produced in several of the soils, but if both nitrites and nitrates are considered, there is an increase in oxidized nitrogen in many of these soils.

Relation to soil type

The work has not progressed sufficiently to discuss the relation of nitrite formation to soil type, but nitrites were produced in large amounts in cultures of the surface soils of such important types as Lake Charles clay, Lake Charles clay loam, and Norfolk fine sand. Nitrites were not produced in the sample of Houston black clay used as a standard (Table 2). The soil types are named in the various tables. Many of the samples are subsoils, but the occurrence of nitrites was observed in connection with work on a study of nitrate formation not planned for a study of nitrites. It must be observed that nitrites did not occur in appreciable quantities in a number of the cultures.

DISCUSSION

It has been shown in the preceding pages that nitrites may occur in considerable amounts in some cultures of soils. This Bulletin only introduces the subject. The practical and scientific importance of nitrites in soils remains to be ascertained, but it is clearly a matter that calls for extensive study under various conditions. Appreciable amounts of nitrites no doubt have been formed in many cultures made in nitrification work previously reported, but since no test was made for their presence, they were not reported. Nitrites may occur in quantity in field soils of particular types, or under special conditions, especially when fertilizers are used, and they may be of agricultural importance. Nitrites may be, under many conditions, as unimportant as they were generally believed to be, but it is obvious that until the subject has been closely invesitgated and their importance or non-importance has been demonstrated under the particular conditions under consideration, it will be imprudent to disregard their possible presence in work on nitrification or nitrates in field soils or cultures. The Texas Agricultural Experiment Station is actively engaged in work on this subject, and no doubt work will be undertaken by others in various sections.

ACKNOWLEDGMENT

Dr. J. F. Fudge made the estimation of many of the nitrites and assisted materially in the adaptation of the method for nitrous nitrogen to the work to be done.

SUMMARY AND CONCLUSIONS

1. Nitrites have been found in large quantity in cultures of soils containing sulphate of ammonia. They have also been found in the cultures which receive no additions of nitrogenous materials.

2. Soils which do not nitrify ammonium sulphate or which have only low nitrifying power for ammonium sulphate, may produce large amounts of nitrites.

3. A low production of nitrates may be accompanied by a high production of nitrites.

4. The relative capacity of a soil to oxidize nitrogen may be considerably larger for nitric and nitrous nitrogen combined than the nitric nitrogen alone.

5. The production of nitrites was small the first week, a little larger the second, and much higher the third and fourth weeks of incubation. The production decreased after 28 days.

6. The most favorable amount of water was 50 per cent of the water capacity of the soil. The production of nitrites as well as nitrates, is usually lower with both larger and smaller amounts of water than 50 per cent.

7. The addition of calcium or magnesium carbonate increased the production of nitrous and nitric nitrogen from ammonium sulphate.

8. Nitrites persisted in the cultures for more than six weeks in considerable amounts. They also persisted practically unchanged for over a week in soil extracts.

9. Nitrites are more stable than they are generally believed to be.

10. Nitrites were present in very small amounts in the samples of field soils and laboratory samples examined.

11. Nitrites were produced from urea in some soils.

12. Nitrites were produced in cultures of important soil types such as Lake Charles clay and Norfolk fine sand.

13. The possible presence of nitrites in cultures and field soils cannot be disregarded and the matter requires further investigation. The work is being continued.

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