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THE SOIL VERSUS THE SOLUTION METHOD AS A MEANS OF STUDYING BACTERIAL ACTIVITIES IN SOIL

BY

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THE SOIL VERSUS THE SOLUTION METHOD AS A MEANS OF STUDYING BACTERIAL ACTIVITIES IN SOIL[1]

By J. E. GREAVES, *Professor of Bacteriology and Biochemistry*, and H. C. PULLEY, *Instructor and Assistant in Bacteriology, Department of Bacteriology and Physiological Chemistry, Utah Agricultural Experiment Station*[2]

INTRODUCTION

The biological decomposition of proteinaceous materials as it occurs in ammonification and nitrification in the soil is a dynamic process. There is not only a constant production of ammonia and of nitric acid but the ammonia is continually being transformed into nitric acid and both products are being synthesized into the bacterial proteins. In addition to these transformations there are constantly going on in the soil many other poorly understood side reactions. It is therefore evident that these processes, as they occur in the soil, are reactions which may reach equilibrum but which never go to completion. The bacteriological methods as used to-day merely measure the accumulation of ammonia or of nitric acid present at a specific moment. It therefore seemed important to determine when this measurement should be made in order to obtain the greatest accumulation of products and at what period the greatest differences are manifest between various soils. The natural processes occurring in the soil were studied both in soil and in solution and the results compared.

COMPOSITION OF SOILS USED

The work was conducted on four different soils: The college farm soil obtained just east of the plant industry building on the campus; Corinne soil; Benson soil; and Richland Acres soil.

The physical analyses of these soils, as determined by the Yoder[3] elutriator, are given in Table 1.

TABLE 1.—*Physical composition of soils used in the tests*

Constituent	College farm	Corinne	Benson	Richland Acres
	Per cent	*Per cent*	*Per cent*	*Per cent*
Sand	36.6	17.5	42.5	4.8
Fine sand	30.6	17.3	20.9	29.6
Coarse silt	18.8	28.5	19.0	27.0
Medium	6.0	15.9	3.8	13.1
Fine silt	2.3	5.3	2.3	10.2
Clay	3.5	7.4	6.1	9.4
Moisture, soluble salts, and loss	2.2	8.1	5.4	5.9

The college farm soil is a highly productive calcareous sandy loam, low in organic carbon and nitrogen but otherwise well supplied with the essential elements of plant food. The Benson soil is a sandy

[1] Received for publication Apr. 18, 1931; issued December, 1931. Contribution from the Department of Bacteriology, Utah Agricultural Experiment Station.
[2] Valuable assistance in the analytical work was rendered by Prof. C. T. Hirst, J. Dudley Greaves, and Milton E. Nelson.

[3] YODER, P. A. A NEW CENTRIFUGAL SOIL ELUTRIATOR. Utah Agr. Expt. Sta. Bul. 89, 47 p., illus. 1904.

loam containing more clay that the college farm soil and sufficient alkali carbonates to render it barren. The Corinne and Richland Acres soils are fine sandy loams, the Corinne soil containing sufficient chlorides and the Richland Acres soil sufficient sulphates to render them barren except to a few salt-tolerant plants. Portions of the college farm soils that had received the following treatments were also used:

2 per cent sodium chloride.
2 per cent sodium sulphate.
2 per cent sodium carbonate.
1 per cent sodium chloride plus 1 per cent sodium sulphate.
1 per cent sodium chloride plus 1 per cent sodium carbonate.
1 per cent sodium sulphate plus 1 per cent sodium carbonate.
⅔ per cent each sodium chloride, sodium sulphate, and sodium carbonate.

Consequently there were used in this work (1) the three native alkali soils; (2) the native productive soil; (3) the native productive soil containing salts in the proportions listed above, and (4) each of the preceding soils after they had been leached with water until the major portions of the soluble salts had been removed.

Before the bacteriological analyses were made the hydrogen-ion concentration of each soil was determined by the quinhydrone method. The average pH values for the various soils are given below:

Soil and treatment	pH
College farm:	
Untreated	8.6
Leached	8.5
2 per cent NaCl	8.7
2 per cent NaCl, leached	8.4
2 per cent Na_2SO_4	8.3
2 per cent Na_2SO_4, leached	8.0
2 per cent Na_2CO_3	10.3
2 per cent Na_2CO_3, leached	9.5
1 per cent each of NaCl, Na_2SO_4	8.3
1 per cent each of NaCl, Na_2SO_4, leached	8.4
1 per cent each of Na_2CO_3, NaCl	8.9
1 per cent each of Na_2CO_3, NaCl, leached	8.9
1 per cent each of Na_2SO_4, Na_2CO_3	9.4
1 per cent each of Na_2SO_4, Na_2CO_3, leached	9.5
⅔ per cent each of NaCl, Na_2SO_4, Na_2CO_3	8.8
⅔ per cent each of NaCl, Na_2SO_4, Na_2CO_3, leached	8.9
Corinne	8.5
Leached	8.5
Richland acres	8.9
Leached	9.0
Benson	10.1
Leached	8.7

The pH value of the native productive soil was 8.6. This was slightly reduced by leaching. The addition of the chloride or carbonate of sodium to the soil increased the pH value whereas the addition of sodium sulphate decreased it. The leaching of the soil which contained the single salts decreased the pH, whereas the leaching of the soil containing a combination of salts usually increased the pH. The pH of the Corinne soil was not very different from that of the normal productive soil, nor was it changed by leaching. The Richland Acres soil had a higher alkalinity, which was further increased by leaching. The Benson soil had an alkalinity approximately equal to the productive soil to which had been added 2 per cent of sodium carbonate. This was greatly reduced by leaching. It is apparent that

there was an appreciable variation in the pH values of the different soils, depending upon the soluble salt that they contained.

TABLE 2.—*Replaceable bases, expressed as milligram equivalents per 100 gm. of soil, in soils used for tests when differently treated*

Soil and treatment	Calcium	Magnesium	Sodium	Potassium	Equivalent of NH_3 required
College farm	23.7	4.5	8.7	0.4	10.6
College farm, leached	21.8	2.1	.6	.4	11.6
NaCl added, leached	24.1	2.6	1.2	.6	10.1
Na_2SO_4 added, leached	26.2	3.8	1.0	.6	11.1
Na_2CO_3 added, leached	21.9	2.7	8.7	.2	8.2
NaCl, Na_2SO_4 added, leached	27.0	2.5	8.7	.1	9.4
Na_2SO_4, Na_2CO_3 added, leached	18.7	2.4	8.7	.3	8.5
Na_2SO_4, NaCl, Na_2CO_3 added, leached	25.4	3.4	1.5	.8	9.6
Corinne, leached	9.1	5.6	5.9	1.0	8.6
Richland Acres, leached	16.2	26.5	7.6	.8	10.2
Benson, leached	17.4	23.6	4.2	1.6	8.5

From the data shown in Table 2 one may gain an idea of the replaceable bases in the various soils. The replaceable bases in the leached soils were in all cases lower than in the nonleached soils. The leaching had carried from the soils appreciable quantities of sodium, potassium, calcium, and magnesium.[4] Yet the leached normal soil had a more active microflora and was more productive than the normal nonleached soil.[5] The soil to which sodium carbonate had been added and which had afterwards been leached had lost considerable of its replaceable calcium, magnesium, and potassium. The native alkali soils were lower in calcium but higher in the other replaceable bases than was the native fertile soil. It is evident, therefore, that these soils varied greatly—chemically, physically, and biologically. The differences in their bacterial activities and productivity were readily measured by the ordinary means.[5]

AMMONIFICATION IN SOLUTION

Preliminary studies were made on the rate of ammonia accumulation in solution. This was done by seeding 1-gm. portions of each of the soils into 100 c. c. portions of peptone solution. These were incubated at from 28° to 30° C. Aliquot portions of the solution were removed daily under aseptic conditions in order not to contaminate the remainder of the media. The ammonia was determined in this sample and the quantity calculated in the original medium. The average results for the various soils for 11-day periods are given in Table 3.

Only small quantities of ammonia accumulated in the media during the first 24 hours, yet there was an appreciable variation, depending upon the specific soil used. Usually at the end of 24 hours there were greater accumulations of ammonia where the leached soil was used than where the unleached soils were used. The mere leaching of the normal soil produced a measurable variation in ammonia accumulation at first but not at the end of 24 hours.

[4] GREAVES, J. E., HIRST, C. T., and LUND, Y. THE LEACHING OF ALKALI SOILS. Soil Science, 16: 407–426. 1923.

[5] GREAVES, J. E. THE MICROFLORA AND THE PRODUCTIVITY OF LEACHED AND NONLEACHED ALKALI SOIL. Soil Science, 23: 271–302. 1927.

TABLE 3.—*Milligrams of ammonia found in peptone after various incubation periods*

[The media had been inoculated with soil containing various soluble salts]

Soil and treatment	Milligrams of ammonia produced in—										
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days
Normal	3.9	21.7	26.5	28.9	52.0	59.8	52.4	58.4	51.5	52.8	47.0
Normal, leached	5.8	20.8	25.9	29.6	50.5	60.6	46.7	55.9	53.7	55.3	46.5
NaCl	3.5	20.8	22.6	28.6	50.8	56.3	57.4	55.6	59.7	55.3	53.2
NaCl, leached	3.3	21.7	22.9	31.6	52.8	63.3	66.1	59.6	58.3	58.0	55.2
Na_2SO_4	3.7	18.4	25.9	32.2	53.0	56.3	56.7	60.9	53.7	54.6	57.8
Na_2SO_4, leached	2.5	19.1	22.2	34.6	48.5	58.0	52.7	60.2	48.3	52.0	46.5
Na_2CO_3	1.1	11.7	16.4	26.2	49.2	62.0	60.1	66.2	57.0	60.6	60.5
Na_2CO_3, leached	2.1	23.7	25.9	37.2	64.5	64.3	70.7	61.6	59.0	57.3	57.2
NaCl, Na_2SO_4	2.5	18.4	27.6	37.9	55.2	60.0	63.4	58.2	51.7	55.3	51.2
NaCl, Na_2SO_4, leached	4.6	24.6	27.6	31.9	60.3	58.6	56.7	56.9	52.3	54.6	52.5
NaCl, Na_2CO_3	2.9	16.2	18.6	27.2	58.5	52.6	70.1	57.6	55.7	52.0	46.5
NaCl, Na_2CO_3, leached	4.9	22.8	20.4	27.2	60.5	57.3	63.4	58.2	63.0	59.3	49.8
Na_2SO_4, Na_2CO_3	1.6	24.6	25.0	34.2	61.8	62.0	60.7	54.9	59.7	55.3	47.8
Na_2SO_4, Na_2CO_3, leached	4.3	22.4	29.6	26.6	65.2	54.6	58.1	58.9	51.7	59.3	46.5
NaCl, Na_2SO_4, Na_2CO_3	2.7	14.2	29.6	27.9	59.8	60.0	54.1	52.9	55.0	48.3	45.2
NaCl, Na_2SO_4, Na_2CO_3, leached	4.2	24.2	30.6	26.9	53.6	62.0	52.7	51.6	49.7	44.0	45.8
Corinne	2.5	16.7	25.6	26.4	63.8	54.6	49.4	59.6	47.0	48.6	43.2
Corinne, leached	4.6	20.2	25.9	27.2	67.8	62.6	56.7	54.9	55.0	60.0	48.5
Richland Acres	2.5	18.4	34.6	30.6	65.2	59.3	57.4	47.6	50.3	45.3	46.5
Richland Acres, leached	4.6	26.6	35.9	30.9	66.5	58.0	60.4	61.6	58.3	47.3	50.5
Benson	1.3	3.1	9.9	14.9	33.8	35.3	41.4	34.2	36.7	42.6	38.2
Benson, leached	2.0	18.2	34.9	27.6	61.8	64.3	53.7	56.2	49.7	52.0	43.2

Considerably more ammonia had accumulated by the end of the second day. However, the variation in the different soils was in the same order, differing only in magnitude. The accumulations of ammonia during the third and fourth days were not as rapid as on the second day. The rate of production reached its height between the fourth and fifth days. After this period there was a slowing down of the reaction. In a few cases there was an actual loss of ammonia during the latter part of the incubation period. The variation due to different soils was in the same order during the first seven days. Beyond this period the results approach a constant. The widest variation in ammonia accumulation in the various soils is found when the determinations are made between the fourth and seventh days. The accumulation of ammonia was greater on the seventh day than on the fourth. Other than the greater accumulation of ammonia, nothing is to be gained by taking the longer incubation period. In each incubation period is manifested the influence of treatment; hence, any of the early periods may be used in studying ammonification provided determinations made only with the same incubation period are compared. It is certain that the ammonifying ferments are not destroyed in soils by as much as 2 per cent of alkali even after long periods, as when the soluble salts are removed or diluted the ferments become active.

NITRIFICATION IN SOLUTION

A study similar to that reported above was made of the nitrifying powers of the same soils. The technic was the same for the ammonification series except that 0.1 per cent of sodium nitrite was used as the substrate to be nitrified. The average results for the sulphate-carrying soils are given in Table 4.

TABLE 4.—*Milligrams of nitric nitrogen found in nitrite media after various incubation periods*

[The media had been inoculated with soil modified by adding sulphates alone and in combination with other salts]

Soil and treatment	Milligrams of nitric nitrogen produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Normal	1.5	1.0	1.8	1.5	3.2	3.3	2.7	2.8	2.5	2.7	4.9	8.9
Normal, leached	1.1	2.0	2.2	1.7	3.7	3.4	3.3	3.6	2.3	3.7	4.9	8.6
Na_2SO_4	1.0	1.4	3.1	3.5	2.5	3.3	3.4	3.0	2.0	2.8	2.5	4.1
Na_2SO_4, leached	.9	1.8	2.3	4.4	3.9	3.4	2.6	3.0	2.1	3.7	3.7	6.0
NaCl, Na_2SO_4	.8	1.6	2.4	2.0	2.8	4.3	3.2	2.8	1.9	2.2	2.5	2.2
NaCl, Na_2SO_4, leached	.8	1.6	2.2	2.1	2.2	3.9	3.4	3.0	2.9	3.9	4.5	5.8
Na_2CO_3, Na_2SO_4	1.1	1.4	2.5	4.1	2.9	3.5	3.4	3.1	2.8	3.5	4.4	6.4
Na_2CO_3, Na_2SO_4, leached	1.1	1.9	2.0	3.4	2.1	2.9	6.3	6.0	4.9	6.5	9.5	20.5
NaCl, Na_2SO_4, Na_2CO_3	1.0	1.2	2.6	1.6	3.1	2.7	5.4	4.8	2.6	3.4	7.0	13.6
NaCl, Na_2SO_4, Na_2CO_3, leached	1.1	1.4	2.3	1.5	2.0	2.4	6.4	3.1	2.8	3.2	4.3	6.5

Soil and treatment	Milligrams of nitric nitrogen produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Normal	12.0	25.5	33.7	41.0	50.0	84.0	84.0	95.0	100.0	104.0	114.0
Normal, leached	16.1	23.3	29.1	41.8	53.0	71.0	89.5	92.5	106.0	103.0	99.0
Na_2SO_4	4.8	8.6	9.5	31.7	51.0	59.0	78.0	95.5	94.0	78.0	95.0
Na_2SO_4, leached	10.0	15.0	17.7	38.0	48.0	53.0	95.0	100.0	97.0	92.0	88.0
NaCl, Na_2SO_4	3.0	2.7	2.3	2.9	3.2	7.4	31.0	50.0	81.0	93.0	97.0
NaCl, Na_2SO_4, leached	14.5	18.0	23.3	37.5	42.5	54.5	69.0	90.0	57.5	88.0	88.0
Na_2CO_3, Na_2SO_4	16.5	21.8	24.7	53.0	62.5	81.0	88.0	103.0	85.5	95.0	95.0
Na_2CO_3, Na_2SO_4, leached	32.8	43.3	44.5	81.5	72.0	79.0	88.0	102.0	95.5	82.0	96.0
NaCl, Na_2SO_4, Na_2CO_3	34.5	50.0	69.0	102.5	85.0	93.5	92.0	94.0	92.5	93.0	93.0
NaCl, Na_2SO_4, Na_2CO_3, leached	19.0	22.1	30.0	68.0	76.0	96.5	96.0	98.0	91.5	91.0	95.0

The accumulation of nitrates during the first 11 days was extremely slow, after which there was an acceleration of the reaction. The rate of the reaction varied widely, depending upon the soil. Where the inoculation had been made with soil containing the three salts, the maximum accumulation of nitric nitrogen was reached between the fifteenth and sixteenth days. After this period there was a decrease in the nitrate content of this solution. This may have been due more to its transformation into protein nitrogen than to a slowing down of the reaction. The corresponding leached soil reached its maximum between the nineteenth and twentieth days. During the first 18 days the soil containing sodium chloride and sodium sulphate caused little accumulation of nitrates, but during the succeeding few days the gains were as great as in the other soils. If one were attempting to measure maximum accumulation of nitric nitrogen, the incubation period should be taken as 21 days, or even longer; whereas, if one wishes to measure the relative speed of nitric nitrogen accumulation and hence the nitrifying efficiency of the various alkali soils it is better to use an incubation period of 15 days.

Two per cent of sodium sulphate in this soil was sufficient to retard the activity but not to destroy the nitrifying ferments, for when conditions were made appropriate the nitrifying ferments commenced to work.

The average results for the chloride-carrying soils are given in Table 5.

TABLE 5.—*Milligrams of nitric nitrogen found in nitrite media after various incubation periods*

[The media had been inoculated with soil modified by adding chlorides alone and in combination with other salts]

Soil and treatment	Milligrams of nitric nitrogen produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Normal	1.5	1.0	1.8	1.5	3.2	3.3	2.7	2.8	2.5	2.7	4.9	8.9
Normal, leached	1.1	2.0	2.2	1.7	3.7	3.4	3.3	3.6	2.3	3.7	4.9	8.6
NaCl, leached	.9	1.7	2.7	1.9	3.8	3.6	2.8	3.2	2.3	2.7	3.9	6.9
NaCl, Na_2SO_4	.8	1.6	2.4	2.0	2.8	4.3	3.2	2.8	1.9	2.2	2.5	2.2
NaCl, Na_2SO_4, leached	.8	1.6	2.2	2.1	2.2	3.9	3.4	4.0	2.9	3.9	4.5	5.8
NaCl, Na_2CO_3	1.3	1.6	2.6	1.9	2.4	3.5	4.0	2.8	1.9	2.2	2.6	2.5
NaCl, Na_2CO_3, leached	.9	1.1	2.6	3.2	3.7	4.3	3.5	3.9	4.2	6.7	13.0	24.2
NaCl, Na_2SO_4, Na_2CO_3	1.0	1.2	2.6	1.6	3.1	2.7	5.4	4.8	2.6	3.4	7.0	13.6
NaCl, Na_2SO_4, Na_2CO_3, leached	1.1	1.4	2.3	1.5	2.0	2.4	6.4	3.1	2.8	3.2	4.3	6.5

Soil and treatment	Milligrams of nitric nitrogen produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Normal	12.0	25.5	33.7	41.0	50.0	84.0	84.0	95.0	100.0	104.0	114.0
Normal, leached	16.1	23.3	29.1	41.8	53.0	71.0	89.5	92.5	106.0	103.0	99.0
NaCl, leached	12.1	20.5	23.8	40.8	50.0	67.0	93.5	98.0	95.0	98.0	98.0
NaCl, Na_2SO_4	3.0	2.7	2.3	3.9	3.2	7.4	31.0	50.0	81.0	93.0	97.0
NaCl, Na_2SO_4, leached	14.5	18.0	23.3	37.5	42.5	54.5	69.0	90.0	87.5	93.0	88.0
NaCl, Na_2CO_3	3.1	2.4	1.8	2.9	5.1	3.1	5.4	10.1	58.0	90.0	91.0
NaCl, Na_2CO_3, leached	48.8	55.5	77.0	110.0	92.0	93.0	96.5	92.0	94.0	95.0	95.0
NaCl, Na_2SO_4, Na_2CO_3	34.5	50.0	69.0	102.5	85.0	93.5	92.0	94.0	92.5	93.0	93.0
NaCl, Na_2SO_4, Na_2CO_3, leached	19.0	22.1	30.0	68.0	76.0	96.5	96.0	98.0	91.5	91.0	95.0

Little nitric nitrogen accumulated during the first 11 days. All solutions contained approximately the same quantities. After this period there was a wide variation and in most cases a rapid accumulation. By the sixteenth day the leached sodium carbonate-sodium chloride soil had accumulated 110 parts per million of nitric nitrogen, whereas the corresponding unleached soil had accumulated only 3 parts per million. It was not until the twentieth day that this soil-inoculated medium commenced to accumulate much nitric nitrogen, but by the twenty-ninth day the accumulation was about as great as in the other soils. It is evident also in this series that the shorter incubation period of 15 days should be used if one wishes to determine the influence of soluble salts on nitrification by the solution method.

The average results for the carbonate-treated soils are given in Table 6.

TABLE 6.—*Milligrams of nitric nitrogen found in nitrite media after various incubation periods*

[The media had been inoculated with soil modified by adding carbonates alone and in combination with other salts]

Soil and treatment	Milligrams of ammonia produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Normal	1.5	1.0	1.8	1.5	3.2	3.3	2.7	2.8	2.5	2.7	4.9	8.9
Normal, leached	1.1	2.0	2.2	1.7	3.7	3.4	3.3	3.6	2.3	3.7	4.9	8.6
Na_2CO_3	1.5	2.5	3.5	3.9	3.1	3.4	3.5	3.6	2.0	2.2	2.3	3.0
Na_2CO_3, leached	.9	1.3	2.0	2.0	2.5	3.0	3.8	3.5	2.8	4.7	8.9	22.2
NaCl, Na_2CO_3	1.3	1.6	2.6	1.9	2.4	3.5	4.0	2.8	1.9	2.2	2.6	2.5
NaCl, Na_2CO_3, leached	.9	1.1	2.6	3.2	3.7	4.3	3.4	3.9	4.2	6.7	13.0	24.2
Na_2CO_3, Na_2SO_4	1.1	1.4	2.5	4.1	2.9	3.5	3.4	3.1	2.8	3.5	4.4	6.4
Na_2CO_3, Na_2SO_4, leached	1.1	1.9	2.0	3.4	2.1	2.9	6.3	6.0	4.9	6.5	9.5	20.5
NaCl, Na_2CO_3, Na_2SO_4	1.0	1.2	2.6	1.6	3.1	2.7	5.4	4.8	2.6	3.4	7.0	13.6
NaCl, Na_2CO_3, Na_2SO_4, leached	1.1	1.4	2.3	1.5	2.1	2.4	6.4	3.1	2.8	3.2	4.3	6.5

Soil and treatment	Milligrams of ammonia produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Normal	12.0	25.5	33.7	41.0	50.0	84.0	84.0	95.0	100.0	104.0	114.0
Normal, leached	16.1	23.3	29.1	41.8	53.0	71.0	89.5	92.5	106.0	103.0	99.0
Na_2CO_3	3.0	3.2	1.8	3.3	2.7	2.8	6.3	3.0	5.0	7.3	28.0
Na_2CO_3, leached	47.5	78.5	84.5	127.0	96.5	95.0	94.5	101.0	96.0	91.0	98.0
NaCl, Na_2CO_3	3.1	2.4	1.8	2.9	5.1	3.1	5.4	10.1	58.0	90.0	91.0
NaCl, Na_2CO_3, leached	48.8	55.5	77.0	110.0	92.0	93.0	96.5	92.0	94.0	95.0	95.0
Na_2CO_3, Na_2SO_4	16.5	21.8	24.7	53.0	62.5	81.0	88.0	103.0	95.5	95.0	95.0
Na_2CO_3, Na_2SO_4, leached	32.8	43.3	44.5	81.5	72.0	79.0	88.0	102.0	95.5	82.0	96.0
NaCl, Na_2CO_3, Na_2SO_4	34.5	50.0	69.0	102.5	85.0	93.5	92.0	94.0	92.5	93.0	93.0
NaCl, Na_2CO_3, Na_2SO_4, leached	19.0	22.1	30.0	68.0	76.0	96.5	96.0	98.0	91.5	91.0	95.0

The soils containing small quantities of sodium carbonate accumulate nitric nitrogen more rapidly than the other soils, but when large quantities of the carbonates are present there is great retardation of the process. The slowest nitrification of the whole series was by the soil containing 2 per cent sodium carbonate, whereas the highest accumulation was by the same soil after leaching. In this series also the maximum variation occurred on the fifteenth day, and by the twenty-first day the variation due to the different salt-treated soils was slight, with the exception of the soil containing 2 per cent sodium carbonate. On the twenty-first day it had accumulated only 5 parts per million of nitric nitrogen; by the end of the thirty-sixth day there were 28 parts per million, thus indicating that the nitrifying ferments are not destroyed by 2 per cent sodium carbonate in soil.

The average results obtained from a study of the three native alkali soils, leached and nonleached, are given in Table 7.

TABLE 7.—*Milligrams of nitric nitrogen found in nitric media after various incubation periods*

[The media had been inoculated with various alkali soils]

Soil and treatment	Milligrams of nitric nitrogen produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Corinne	1.2	1.8	2.1	2.3	4.2	2.4	5.7	2.5	2.1	2.2	2.6	2.5
Corinne, leached	1.8	1.6	2.0	3.1	2.5	2.4	5.2	3.3	2.3	3.0	4.0	4.9
Richland Acres	1.4	1.1	2.7	2.0	2.7	2.3	8.7	3.7	2.7	3.9	5.8	8.2
Richland Acres, leached	1.3	1.2	2.5	3.3	2.6	3.0	4.9	4.4	3.0	4.7	6.4	10.5
Benson	1.4	1.4	2.4	3.3	4.3	3.2	5.3	3.5	2.7	2.5	2.4	4.0
Benson, leached	1.5	1.4	2.8	1.9	2.6	2.7	9.0	5.7	3.1	2.8	3.1	2.8

Soil and treatment	Milligrams of nitric nitrogen produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Corinne	3.1	2.1	1.8	2.8	2.9	5.1	16.0	27.0	32.0	84.0	94.0
Corinne, leached	9.5	11.2	14.8	37.0	45.5	73.0	93.8	89.0	90.0	96.0	94.0
Richlahd Acres	17.5	19.8	31.3	49.5	57.5	89.5	97.0	100.0	93.5	93.0	90.0
Richland Acres, leached	24.3	25.5	37.8	59.5	67.0	89.5	96.5	92.0	91.0	93.0	90.0
Benson	3.7	4.2	2.1	3.1	2.4	2.5	3.5	2.6	5.0	55.0	91.0
Benson, leached	4.2	3.0	3.0	5.5	3.4	6.9	9.7	13.9	17.4	68.0	93.0

During the first 12 days little nitric nitrogen accumulated in any of the media. However, a rapid accumulation occurred between the twelfth and eighteenth days in the Richland Acres soils, leached and nonleached, and in the Corinne leached soil. The Corinne leached soil accumulated small quantities of nitric nitrogen during the first 18 days, but by the end of the twenty-ninth day the media into which it had been inoculated was carrying about as much nitric nitrogen as did the other media. The Benson leached and nonleached soil caused slight accumulation of nitric nitrogen the first 18 days, but by the thirty-sixth day the accumulation was as great as with the other soils. In the native alkali soils the greatest variation of nitric nitrogen in the solutions occurred later than the fifteenth day.

It is evident from the results on all the soils that the greatest variation in accumulation of nitric nitrogen in the solutions inoculated with the various soils occurs between the 15 and 18 days. By the thirty-sixth day the quantity of nitric nitrogen in the media treated with the various soils approaches a constant. If one uses the shorter incubation periods, it is evident that there is a wide variation in the nitric nitrogen accumulation in liquid media. This varies with the specific soil which has been used as the inoculum. It is further evident that soluble salts stimulate or retard nitrification as measured by this method, the extent depending upon the specific salt, its combination with other salts, and its concentration. Even large quantities of the soluble salts may occur in soils over long periods without destroying the nitrifying ferments. The salts inhibit their actions and when diluted the action of the nitrifying ferments is manifest.

AMMONIFICATION IN SOIL VERSUS IN SOLUTION

The ammonifying powers of the various soils were determined both by the soil and the solution methods. The technic for the solution method was the same as that previously given. The incubation period was seven days. The substrate to be ammonified was peptone. The tests in soil were made by mixing 2 gm. of dried blood with 100 gm. of soil in tumblers, making the moisture up to 18 per cent, covering with Petri dishes and incubating for seven days at 28° to 30° C. The ammonia was then determined in the regular way. The average results for both the solution and the soil methods are given in Figure 1.

The native productive soil was a highly calcareous loam which became more productive on leaching. The addition of soluble salts to it had rendered it barren. The three native alkali soils were barren. The leaching of the soils rendered a few productive, and in

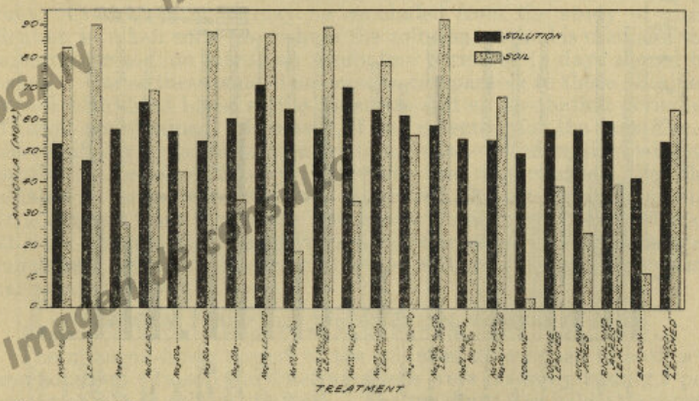

FIGURE 1.—Milligrams of ammonia found in soil and in liquid media inoculated with the same soils

some cases the leached soils produced larger crops of crimson clover than the native nonalkali soil. The increase usually occurred where the sulphates had been added and was due in part to the addition of sulphur to this soil low in sulphur.[6] There is a close correlation between the productivity of the soils and their ammonifying powers, as measured by the soil-tumbler method. If the ammonia accumulations in the soil are compared with those in the solution, a direct correlation is found in only three cases; these are leached soils. The soluble salts depress in the soil; where diluted, however, as in the solution, they often stimulate. After the salts have been leached from the soil and the soil inoculated into the solution the concentration of the salt is usually too low to stimulate. The Corinne soil, leached and nonleached, is nonproductive. The nonleached soil produces little ammonia in the soil, whereas in the solution there is a large accumulation. The leaching increases the accumulation both in the soil and in the solution. One might conclude from the results obtained

[6] GREAVES, J. E., and GARDNER, W. IS SULFUR A LIMITING FACTOR OF CROP PRODUCTION IN SOME UTAH SOILS? Soil Sci. 27: 445-457, illus. 1929.

on this soil by the soil-tumbler method that the ammonifying organisms are absent, which is certainly erroneous, as is seen by an examination of the results obtained by the solution method. Consequently, it may be concluded that where one desires to gain an insight into the relative productivity of a soil the soil-tumbler method is greatly to be preferred to the solution method for testing the soil. If one desires to determine the presence or absence of the ammonifying ferments the solution method is to be preferred to the soil-tumbler method.

NITRIFICATION IN SOIL VERSUS IN SOLUTION

Nitrification both in soil and in solution was determined on all the soils previously described. The technic was the same as that described under ammonification except that in solution the substrate to be nitrified was sodium nitrite and the incubation periods in the solutions were 15 and 21 days, whereas in the soil an incubation period of 21 days was used. The average results for soil and solution are given in Figure 2.

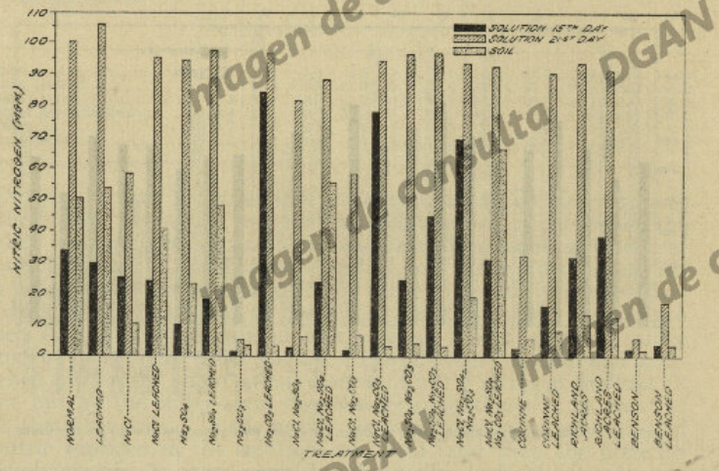

FIGURE 2.—Milligrams of nitric nitrogen found in soil and liquid media inoculated with the same soil. The incubation periods were 15 and 21 days for the solutions and 21 days for the soil

The determinations made in soil, with four exceptions, yielded greater accumulations of nitric nitrogen in the leached than in the nonleached soil, the exceptions being where the carbonate was present. The carbonate left the leached soil in a bad physical condition, the soil structure having been broken down. There was in many cases an appreciable stimulation of nitrification due to the soluble salts. From these results one might erroneously conclude that sodium carbonate had destroyed the nitrifying ferments. However, their activities were only inhibited by the soluble salts present, as is seen from the results obtained in solution. Hence, in dilute solutions they often act as stimulants. The stimulation may be real or apparent: (1) The salts may accelerate the activities of the ammonifers; consequently, ammonia would be produced more rapidly for the use of the nitrifers. This may occur where the dried blood is the substrate decomposed but not where sodium nitrite was used. (2) They may depress denitrification or the transformation of the nitrates into protein nitrogen. Inasmuch as tests of these processes indicate that they are accelerated and not retarded by weak concentrations of these soluble salts it is not probable that the accumulation is due to this factor. (3) They may stimulate more rapid nitrification. This last possibility appears to be the correct interpretation of the data. The accumulation of nitric nitrogen in the solution during 21 days, with three exceptions, is greater in the leached than in the nonleached soils. In the soil there is a stimulation in three cases; in the solution in one case.

On the fifteenth day of incubation all except two of the solutions which had been seeded with the leached soils showed a gain in nitric nitrogen over the nonleached soils. The spread between the various soils is more pronounced on the fifteenth day than it is on the twenty-first. Consequently, it may be concluded from the study of nitrification in alkali soils that where the solution method is used and the substrate is sodium nitrite an incubation period of 15 days should be taken. Under these conditions, results comparable to those obtained in soil with dried blood as the substrate and an incubation period of 21 days are obtained. However, if one is testing for the presence of the nitrifying ferments, it is better to use the solution method with an incubation period of 21 days; otherwise, one may conclude that the alkalies in some instances have destroyed the nitrifying ferments. It is evident from the results obtained with an incubation period of 21 days that the nitrifying ferments will tolerate over long periods as much as 2 per cent of sodium chloride, sodium sulphate, or sodium carbonate in a soil.

NITROGEN FIXATION IN SOLUTION VERSUS IN SOIL

The nitrogen-fixing powers of the soil were determined in both soil and solution. In soil the determinations were made by adding 2 per cent mannite to soil in tumblers. The moisture was held at approximately 20 per cent. The cultures were incubated at 28° to 30° C. for 21 days and the nitrogen determined.

In solution, the determinations were made by seeding 1-gm. portions of the various soils into 100 c. c. portions of Ashby solution containing 2 per cent mannite. These cultures were incubated for 21 days and the nitrogen determined.

The average results for soils and solutions are given in Figure 3. There is little correlation between the results obtained in solution and those obtained in soil. In solution the native leached soil and the sodium-sulphate leached soils gained more nitrogen than did the native soil. All soils showed slight gains in nitrogen, thus demonstrating the presence of nitrogen-fixing organisms. With few exceptions the leached soils gained larger quantities of nitrogen than did the nonleached soils. In most cases the fixation was greater in the soil than in the solution; hence, care should be used in comparing nitrogen fixation as measured by the two methods. Many of the salts stimulate fixation in the soil, and in a few cases, as for instance in the Benson soil, the gain is extraordinary; this property has been repeatedly observed in a study of this soil. The Richland Acres soil makes

no gains in nitrogen when incubated directly, but when inoculated into Ashby solution gains appreciable quantities of nitrogen.

SUMMARY

An extensive bacteriological study was made on soils varying widely in physical, chemical, and biological properties. One of the soils used was a highly productive calcareous loam. This was employed in its native condition and also after it had been made unproductive by the addition of three salts, both singly and in different combinations of twos and threes. Still other samples of the native and variously treated soils were leached and then used in the experimental work. With these artificially prepared soils were used three native alkali soils, both leached and unleached. The hydrogen-ion concentration of the different soils varied from pH 8.0 to 10.3; the replaceable bases—calcium, magnesium, sodium, and potassium—varied

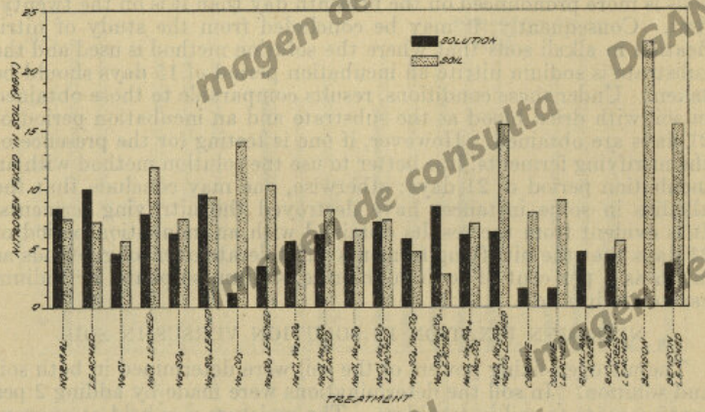

FIGURE 3.—Milligrams of nitrogen fixed in soil and in Ashby media inoculated with the same soils. The incubation periods were 21 days

widely in the different soils and to a lesser extent in the variously treated soils.

When the soils were inoculated into peptone solution the production of ammonia varied with the specific soils and the incubation period. It was slow, then rapid, followed by a slowing down of the process and a decrease in ammonia, probably due to its transformation into protein nitrogen. The ammonia accumulation varied, depending upon the specific soil, its treatment, and the incubation period. It would appear that an incubation period of from one to seven days may be used; however, results obtained only with similar incubation periods are comparable.

The greatest accumulation of nitric nitrogen occurred between the nineteenth and twenty-first days, but the greatest variation in nitric nitrogen accumulation occurred on the fifteenth day. Consequently, if one wishes to obtain the maximum accumulation of nitric nitrogen in solutions an incubation period of 21 days should be used, but if one wishes to learn the relative rate of nitric nitrogen accumulation in alkali soils an incubation period of 15 days should be used.

By using an incubation period of 15 days one gets a fair correlation between solution and soil method. One may at times conclude from results obtained that soluble salts in concentrations of 2 per cent destroy the nitrifying ferments, a conclusion that is shown to be erroneous when the results obtained in solutions are examined. The ammonifying, nitrifying, and nitrogen-fixing ferments withstand 2 per cent of sodium sulfate, sodium carbonate, or sodium chloride in soil for long periods.

All soils studied showed gains in nitrogen, both by the soil and by the solution methods. However, the gains were usually greater when the determinations were made by the soil-tumbler method than when they were made by the solution method.