"gravimetric method," a second sample was measured in a sucrose pipette and the analysis completed as usual, and a third sample was analyzed by the new method. The results obtained are shown in the following table :

TABLE IV.—COMPARISON OF METHODS OF INDIRECT ANALYSIS.

Juice taken. Grams.	Liquid added. cc.	Sugar by new method. Per cent.	Sugar by gravimetric method. Per cent.	Sugar by pipette method. Per cent.
21.78	65.12	9.75	9.8	9.8
29.92	89.46	12.8	12.8	12.65
22.04	65.97[1]	6.35	6.4	6.3
20.02	59.84	10.9	10.9	..
28.36	84.50	9.35	9.35	9.35
23.91	71.49	10.95	11.0	10.95
25.00	74.75	11.10	11.15	11.05
25.13	75.14	13.55	13.65	13.60

These few results are sufficient to show that the new method is applicable to indirect analysis. In point of accuracy it does not possess any great advantage over the sucrose pipette method if the latter is carefully carried out. It has the advantage that no specially constructed apparatus is necessary and it requires slightly less time for its manipulation than do any of the other methods.

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THE RATE OF NITRIFICATION OF SOME FERTILIZERS.

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THE value of any fertilizer depends on its availability to the plant, that is, the readiness with which it can be absorbed directly by the plant, or converted into forms which can be assimilated. Nitrogen can be assimilated by plants directly in four forms; *viz.*:—(1) free nitrogen; (2) as certain organic compounds ; (3) as ammonium salts ; (4) as nitrates.

Free nitrogen can be assimilated from the air by a class of plants with the aid of organisms living in nodules on their roots. This method of assimilation is confined to the leguminosae, which includes clover, peas, beans, the peanut, vetch, etc.

[1] Used water factor 90 per cent. in calculation.

Some organic compounds, such as urea, glycocoll, leucin, tyrosin, asparagin and acetamide, may be taken up directly by plants, and serve to nourish them. All of these compounds may occur in the soil. Urea is found in urine, asparagin in plants, and asparagin and tyrosin are often produced by the decay of animal or vegetable matter in the soil. All nitrogenous organic compounds applied to the soil change to nitrates with greater or less rapidity, and in this form are readily taken up by the plant.

Ammonium salts also can be assimilated by plants. German millet, golden millet, watermelons, corn, common sorrel, and other plants, seem to be able to assimilate ammonium salts directly. Ammonium salts also are converted to nitrates when placed in the soil.

While some plants can assimilate free nitrogen, others organic compounds, and still others nitrogen in the form of ammonia, nitrates appear to be the form in which nitrogen is taken up with the greatest readiness by most plants. It is also the form which all nitrogen compounds finally assume when placed in the soil.

When combined nitrogen, in whatever form of combination, is placed in the soil, it is converted into ammonium salts, nitrites, and finally nitrates, with greater or less speed, depending on the form of combination, the temperature, condition of the soil, etc., provided certain living organisms are present (and they usually are). If in any given soil, we determine the relative rate with which nitrogenous fertilizers which cannot be utilized directly by the plant, are converted into nitrates, it should throw some light upon the relative values to plants of those particular fertilizers. This is the object of the work which will be described in the following pages.

HISTORICAL.

Müntz and Girard[1] have determined the relative rate of nitrification of some fertilizers. A small quantity of the fertilizer was intimately mixed with natural soil, and kept at the temperature of 15°–25° C., properly moistened, and at the end of a given period leached with water, and the nitrate determined in the extract. The nitrate existing in the soil at the beginning of the experiment was previously determined. The time was 30, 32, and 39 days for different sets. The nitrogen converted into nitrates, and the nitrogen recovered from the soil by horse-tooth corn in two years, is shown in the following table:

[1] *Central-Blatt agr. Chem.*, **20**, 656 (1891) abs.

	Nitrified in 30 days. Per cent.	Recovered by corn. Per cent.
Ammonium sulphate	75.0	76.7
Dried blood	72.4	55.0
Roasted horn, fine	71.0	60.1
Flesh meal	70.4	....
Horn trimmings, fine	55.5	53.3
Poudrette, rather coarse	18.1	14.9
Roasted leather, fine	11.6	38.3
Leather chips, raw	0.4	....

In another series, the order of nitrification was as follows: Bat guano, dried grasshoppers, dried cockchafers, flesh meal, dried blood (the substance nitrified to the greatest extent being given first). There is very little difference between the three substances named last. The nature of the soil has a great influence on the change. Nitrification was most active in a light soil from Joinville (used in the experiment referred to above), then in a garden soil, then in a chalky soil, then in a marled moor soil. Very little nitrification occurred in a very calcareous clay, except with cow manure, and yellow lupines, which loosened their texture; and none in an acid moor soil, with the same two exceptions.

P. Bonâme[1] determined the nitrates in the drainage water from a sandy soil deficient in lime to which fertilizers had been added. The order of nitrification at the end of the first month was found to be,—fish guano (most rapid), blood, fertilizer, oil cake, and ammonium sulphate. When calcium carbonate was added, nitrification took place more rapidly, but the order was still dried blood, oil cake, ammonium sulphate (see Table I).

TABLE I.

Nitrate nitrogen in 100 grams soil (in mg.)

	One month.	Two months.	Three months.
Soil	4.2	5.0	5.0
Soil and ammonium sulphate	22	29	35
Soil and dried blood	66	74	85
Soil and oil cake	59	82	95
Soil and fish guano	74	110	113
Soil and calcium carbonate	6.2	7.3	6.0
Soil, calcium carbonate, and ammonium sulphate	75	133	186
Soil, calcium carbonate, and dried blood	123	151	159
Soil, calcium carbonate, and oil cake	97	139	137

[1] *Expt. Sta. Record*, **9**, 732 abs.

It will be noted that where calcium carbonate was not used, nitrates were formed more slowly through the entire period from ammonium sulphate than from the organic substances used. When calcium carbonate was added, the quantity of nitrates produced for the first and second months from ammonium sulphate was smaller than from the organic substances. At the end of the third month, a larger quantity of nitrates was formed from ammonium sulphate than from organic materials.

EXPERIMENTAL.

Effect of Dilution of Soil.—The effect of ratio of soil to fertilizer was studied in some preliminary experiments. 3000 grams of a sandy soil from a pasture, which had been sifted through a 6-mesh sieve, was mixed well with the quantity of dried blood containing 1.0, 0.5, 0.25, gram nitrogen, and the mixtures placed in a dark closet for fourteen days. They were watered at suitable intervals, endeavoring to maintain the original 10 per cent. of water. The temperature was about 27° C. The nitrates were leached out at the end of the period, and their quantity determined by the Tiemann–Schulze method. Results are given in Table II.

TABLE II.

	Dilution.	Nitrates. Gram.	Nitrified. Per cent.
Soil	....	0.0963	....
Soil and 1.0 gram nitrogen	$^1/_{3000}$	0.4564	36.0
Soil and 0.5 gram nitrogen	$^1/_{6000}$	0.3626	52.3
Soil, 0.5 gram nitrogen, and 1.785 gram calcium carbonate	....	0.5043	81.6
Soil and 0.25 gram nitrogen	$^1/_{12000}$	0.2354	55.6

The rapidity of the nitrification is influenced very decidedly by the dilution, and increased by calcium carbonate from 100 to 156. Thirty pounds of nitrogen per acre is a liberal application for a fertilizer. Assuming that the mean weight of a cubic foot of soil is 80 pounds, and that the soil is cultivated to the depth of 6 inches, then the dilution of the nitrogen applied as a fertilizer is $^1/_{57000}$, which is much greater than in any of the above cases. But it must be remembered that a fertilizer is never mixed intimately with the soil, and is often in lumps, so that the actual soil surface in contact with the fertilizer is probably much less than $^1/_{12000}$. This would be particularly true with materials like dried blood, which are insoluble in water. Soluble fertilizers, like ammonium sulphate, would diffuse until they become fixed, or the soil water becomes of a uniform composition; the diffusion of salts in a soil must be a very slow process.

RATE OF NITRIFICATION.

The experiments to determine the relative rate of nitrification were carried out as follows: The fertilizing materials were those sent out by the referee of the Association of Official Agricultural Chemists for 1900, to test the methods for determining the availability of nitrogen. A sandy clay soil from a pasture was sifted through a coarse sieve (6 meshes to the inch), and a quantity of material equivalent to 0.6 gram nitrogen was intimately mixed with 1000 grams of the soil. The soil was then placed in precipitating jars, and kept in a dark closet, enough water being added to raise the percentage from 6.3 to 11.6 per cent. At suitable periods, three of the jars were weighed, and the estimated loss of water was replaced in all the jars. The temperature was 28°–30° C., and the time was three weeks. When calcium carbonate was added, the amount was exactly sufficient to combine with the nitrogen of the fertilizer if the entire amount were converted to nitric acid. At the end of the experiment, the nitrates were leached out, and the amount determined by the Tiemann-Schulze method. The amount of nitrates found in a blank experiment was deducted from the total. The results are given in table III.

On account of the surprisingly small percentage of ammonium sulphate nitrified in the first series, the experiments with cottonseed meal and ammonium sulphate were repeated, the time being twenty-six days, the temperature 23°–26° C., and the sample moistened as before. The soil was taken from the same pasture as in the first series, but differed from it somewhat, as is shown by the fact that it contained 0.1641 gram nitrogen as nitric acid per kilogram, whereas the former contained only 0.0595 gram.

RATE OF NITRIFICATION AND AVAILABILITY OF NITROGEN.

We have selected, and give below (Table III), the results obtained by vegetation tests with oats and Hungarian grass by Jenkins and Britton[1] and those obtained by Bizzell in the laboratory of this Station with the pepsin-hydrochloric acid method,

[1] *Conn. State Station Report* 1897, 357.

and the neutral permanganate method, the materials being those used in these nitrification experiments.

TABLE III.

	Rate of nitrification				Availability.		
	Without		With $CaCO_3$.		Soluble		Vegetable
Series I.	Per cent.	Rank.	Per cent.	Rank.	$KMnO_4$.	Pepsin.	Test.
Dried blood	34.8	100	54.9	100	94.4	94.7	73.3
Cottonseed meal	33.9	97	54.8	100	91.1	91.1	64.8
Dried fish	30.3	87	46.5	85	88.7	67.3	63.9
Tankage	26.2	75	34.8	63	88.3	56.4	49.4
Bat guano	22.4	64	35.8	65	75.1	56.4	..
Bone	18.9	54	16.6	30	64.2	92.3	16.7
Bone (six weeks)	21.7	..	17.4	..	..	..	..
Ammonium sulphate	1.3	4	31.1	55	100	100	..
Sodium nitrate	..	..	..	..	100	100	100
Series II.							
Cottonseed meal	26.7	..	..	..	..	..	..
Ammonium sulphate	3.4	..	32.6	..	.	..	..

The order of availability as determined by the neutral permanganate method, and by the vegetation experiments, is the order of nitrification, except in the case of ammonium sulphate. The pepsin-hydrochloric acid method places bone next to blood, and above cottonseed meal, where it does not belong.

The mechanical condition of the material would, of course, have great effect on the rate of nitrification. It is quite possible that the organic fertilizers contain two or more nitrogen compounds of different degrees of susceptibility to the nitrifying organisms. Bone was nitrified to the extent of 18.9 per cent. in three weeks, and only 21.7 per cent. in six weeks.

EFFECT OF CALCIUM CARBONATE.

Taking the quantity of nitrates formed without the presence of calcium carbonate as 100, the quantity formed with it present was, with dried blood, 158; cottonseed meal, 162; dried fish, 153; tankage, 133; bat guano, 160; bone (three weeks), 88; bone (six weeks), 80; ammonium sulphate, 2390; ammonium sulphate (Series II), 959. This effect may depend on the quantity of bases present in the material. The rate of nitrification of bone, which contains large quantities of calcium salts, is actually decreased by the addition of calcium carbonate while that of ammonium sulphate is increased enormously. These results show the beneficial influence of lime in rendering nitrogenous fertilizers available, and explain in part why lime is so beneficial to many crops.

When ammonium sulphate is used as a fertilizer, it would be advisable to add calcium carbonate at the same time, in many cases.

NITRIFICATION OF AMMONIUM SULPHATE.

As regards ammonium sulphate, in a soil deficient in lime, it is nitrified less readily than any other of the fertilizing materials tested. In the soil to which calcium carbonate had been added, the rate of its nitrification still falls below that of cottonseed meal, blood, and dried fish, and was in one series less, the other greater, than tankage and bat guano, but the average was below. In Bonâme's experiments ammonium sulphate was nitrified during the first and second months less rapidly than any of the other fertilizing materials used (blood, oil cake, guano) whether calcium carbonate was added or not. On the contrary the experiments of Müntz and Girard (presumably in a soil containing calcium carbonate) place ammonium sulphate at the head of all the fertilizing materials tested (blood, flesh meal, poudrette, roasted leather, leather chips).

There are three possible ways to account for the slow rate of nitrification of ammonium sulphate.

1. Ammonium sulphate may hinder the action of the nitrifying organism. The soil in question contained 2.5 grams ammonium sulphate dissolved in 100 grams soil water. It is known that various salts will retard the nitrifying activity of the organisms if present in too large quantity. Deherain found that common salt began to be harmful when more than 0.1 per cent. of the weight of the soil was added, and with larger quantities nitrification almost ceased. Large additions of sodium nitrate also decrease the rate of nitrification.

This explanation will not account for the beneficial action of calcium carbonate, for if double decomposition takes place, the ammonium carbonate formed is more of a hindrance to the germs than the ammonium sulphate.

The assumption that ammonium sulphate hinders the action of the nitrifying organism would explain the low rate of nitrification of ammonium sulphate that we have obtained. It would also explain the results of Bonâme (already cited), according to which ammonium sulphate is nitrified very slowly indeed the first and second months, and very rapidly the third. In direct contradiction to the above hypothesis, however, would stand the experiments of Müntz and Girard, who found that, in thirty days, ammonium sulphate was nitrified to a greater extent than dried blood, etc., and those of Th. Schloesing.[1] The latter found that at the end of fifty-six days ammonium chloride added to a soil at the rate of 3.58 grams per kilo (1.8 grams per 100 cc. of soil water) was almost completely nitrified, and the same occurred with ammonium sulphate at the rate of 2.7 grams per kilo (1.4 grams per 100 cc. soil water) in twenty-two days, and ammonium carbonate at the rate of 0.53 gram ammonia per kilo in twenty-eight days. The soil contained 19.4 per cent. water.

These difficulties might be explained by supposing that the ammonium sulphate affects the nitrifying germs less in some soils than in others, either on account of the different character of the soils (power of fixing ammonia, etc.) or the presence of different kinds of nitrifying organisms.

If the ammonium sulphate is detrimental to the nitrifying organisms, the same kind of action would take place when it is used as a fertilizer though perhaps to a less degree. Each lump of the salt would become a center from which would diffuse a solution of ammonium sulphate, detrimental to the nitrifying organisms. The time required for this unfavorable condition to disappear, would depend on the rate of diffusion of the salt, soil moisture, rainfall, etc.

2. The second explanation for the slow rate of nitrification of ammonium sulphate compared with the other materials, is that the nitric and sulphuric acids formed are detrimental to the nitrifying organisms, being only neutralized in part by the bases of the soil. When calcium carbonate is added, it neutralizes the acids, with consequent acceleration of the change. This explanation is probably applicable, but does not explain all the facts, for if so, the addition of calcium carbonate would remove the unfavorable conditions, and place ammonium sulphate at the head of the list, which it does not do.

3. The third explanation is, that different soils contain different nitrifying organisms, some of which convert organic matter directly to nitrites, while others change ammonium salts to

[1] *Central-Blatt agr. Chem.* **19**, 1 (1890) abs.

nitrites more readily. The nitrites are then converted to nitrates. In soils containing the first kind of organisms, and few of the second, organic matter would be converted to nitrites more rapidly than ammonium salts would be, as was the case in the experiments of Bonâme, and those here described. In soils in which the second class of organisms predominate, ammonium salts would be nitrified more rapidly than organic compounds. This hypothesis would explain all the experiments here cited.

It appears very probable that all three of the explanations given above apply, and that all three are in operation, one exerting a greater influence in some soils than others. It is the purpose of this Station to continue the experiments on nitrification, with a view to test all the problems that may arise.

CONCLUSIONS.

1. The nitrification of blood takes place more rapidly when it is mixed with a large quantity of soil, than with a small quantity.

2. The order of nitrification in the soil used was, dried blood (most nitrified), dried fish, tankage, bat guano, bone, ammonium sulphate. Excluding the ammonium sulphate, this is the order of availability, as measured by vegetation tests, and solubility in potassium permanganate.

3. When calcium carbonate was added to the soil, the nitrification was greatly accelerated, and the order became, dried blood, cottonseed meal, dried fish, bat guano, tankage, ammonium sulphate, bone.

4. When ammonium sulphate is used as a fertilizer, in most cases it would be advisable to add calcium carbonate in some form also.

5. The low rate of nitrification of ammonium sulphate is probably due to the presence of organisms which nitrify organic compounds in preference to ammonium salts. The presence of the ammonium sulphate may also hinder the activity of the nitrifying organisms. The acids formed may also be a hindrance when no base is present to neutralize them. All three of these causes may be in operation at the same time.