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# The Nitrification of Pyridine, Quinoline, Guanidine Carbonate, etc., in Soils

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# THE NITRIFICATION OF PYRIDINE, QUINOLINE, GUANIDINE CARBONATE, ETC., IN SOILS.[a]

By
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Associate Agronomist, Alabama Polytechnic Institute.

## INTRODUCTION

In a previous publication it was shown that certain organic nitrogenous compounds, which had been described by others as being toxic to plants in water cultures, proved to be decidedly beneficial to crops in soil cultures (5)[b]. The yields obtained with both oats and corn were nearly as great with pyridine or quinoline as the source of nitrogen as when nitrate of soda was the source. From the plant growth obtained, it was very evident that these compounds were supplying nitrogen to the crops grown. Either these plants were using the compounds directly, or the compounds were being changed in the soil to some available form. Since most of the compounds studied in the work referred to above had already been proved to be toxic to higher plants in solution cultures, it was concluded that the substances were most likely decomposed to a simple form available to crop plants. If decomposition proceeded very far, in all probability, the nitrogen applied to a soil in the form of pyridine, or similar substances, would later be found as nitrates. It is the purpose of this paper to set forth the results from experiments to determine if such compounds are nitrified in soils.

## Review of Literature

So far as the writer has been able to find from the literature available, the only instance where actual nitrification of the compounds used in this investigation has been observed is that recorded by Buddin. In studies on partial sterilization of soils by means of antiseptics Buddin (2) used pyridine, along with a number of other compounds. The action of pyridine was quite different from that of most other compounds

(a) Published as a continuation of experiments reported in Alabama Experiment Station Bulletin No. 191.
(b) Reference is made by number to "Literature cited" P. 81.

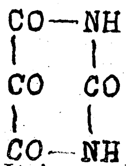
used, in that from its use there resulted an enormous increase in the number of bacteria present and a very great increase in the ammonia and nitrate content of the soil. The increased amounts of ammonia and nitrate were roughly proportional to the increased applications of pyridine, up to 7.9 gms. of pyridine per kilo. of soil.

### CHEMICALS USED.

The dihydroxystearic acid used in the work was made by Kahlbaum; the salicylic aldehyde was a synthetic product obtained from Eimer & Amend. All others were Merck's products.

According to results obtained in solution cultures, pyridine, quinoline, piperidine, guanidine carbonate, naphthylamine, and alloxan are toxic to wheat seedlings. Nucleic acid and asparagine are beneficial under the same conditions.

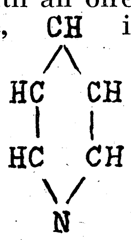
A brief description of the chemicals used, as taken from Bernthsen (1), Holleman (6), and Jones (7), follows:

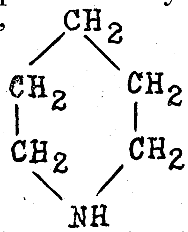
Alloxan,  is a decomposition product of uric acid. It is readily soluble in water, and is of a strong basic nature.

Asparagine, $CO_2H.\ CH\ (NH_2).CH_2.\ CO.\ NH_2$, is an acid amide which is widely distributed in the vegetable kingdom.

Guanidine is a colorless, crystalline compound with strong basic properties. The carbonate, $(CN_3H_5)_2,H_2CO_3$, crystallizes in quadratic prisms. The base is readily hydrolysed, first to urea and ammonia, and finally to ammonia and $CO_2$.

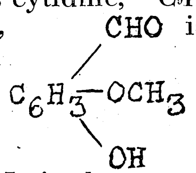
Naphthylamine, $C_{10}H_7\ .NH_2$, is a solid, basic compound, with an offensive odor.

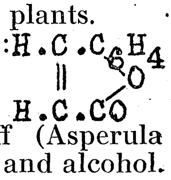
Pyridine,  is regarded as benzene with one CH group placed by a N-atom. Pyridine acts as a base, forming salts with acids. It is a colorless liquid of great stability, being unattacked by boiling nitric acid, or chromic acid. Pyridine may be reduced to piperidine, and piperidine may be oxidized to pyridine.

Piperidine,  is present in pepper in combination as piperine. Piperidine is a colorless liquid with characteristic odor, and strong basic properties.

Quinoline, $C_9H_7N$, is a colorless liquid with peculiar odor. It yields salts with acids, acting like a tertiary base. It is considered to carry a benzene and a pyridine nucleus.

Nucleic acid is a complex substance. Yeast nucleic acid has been given the formula $C_{36}H_{55}N_{15}P_4O_{32}$. It yields on hydrolysis, guanosine, $C_{10}H_{13}N_5O_5$; adenosine, $C_{01}H_{13}N_5O_4$; cytidine, $C_9H_{13}N_3O_5$; and uridine, $C_9H_{12}N_2O_6$.

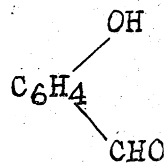
Vanillin,  is considered to be a hydroxaldehyde. It is the aromatic principle of vanilla, and is found in a number of different kinds of plants.

Cumarin has the following composition: 

It is the aromatic principle of woodruff (Asperula odorate). It is soluble in hot water, ether and alcohol.

Pyrogallol is a trihydric phenol to which has been given the formula $C_6H_3(OH)_3$. In alkaline solutions it is a strong reducing agent.

Salicylic aldehyde occurs in oil of spirea; its formula is given as follows:



Dihydroxystearic acid as isolated from soils by Schreiner and Shorey (9) has been given the formula

$$CH_3.\ (CH_2)7.CHOH$$

$$COOH.\ (CH_2)7.CHOH$$

and melting point of 99 degrees. The compound used in this work had a melting point of about 121 degrees, and since there is no known dihydroxystearic acid with this melting point, evidently the product used was impure, and of unknown identity.

## EXPERIMENTAL WORK

### Methods

All of the experiments on nitrification of organic compounds herein reported were conducted in ordinary glass tumblers, using one hundred grams of air-dried soil in each tumbler. Before weighing out the soil, each lot to be used was thoroughly mixed so as to afford uniform samples. Unless otherwise stated, one gram of calcium carbonate, one tenth gram (or one tenth of a cubic centimeter in case of liquids) of the substance to be used was thoroughly mixed with the one hundred gram portions of soil in the tumblers. Sufficient distilled water was added to bring the soil to approximately optimum water content. The tumblers were then weighed, covered and set away in a dark closet in the laboratory. From time to time the tumblers were reweighed, and the loss made up with distilled water.

The phenoldisulphonic acid method was used for all nitrate determinations. Where large quantities of nitrates are present, the error involved in this method is probably great. But the data obtained are thought to be accurate enough to give reliable indications as to the nitrifications of the compounds studied. The contents of each tumbler were washed into a quart jar with 500 c.c. of a solution containing four c.c. of saturated potassium alum, one c.c. of formaldehyde, and four hundred and ninety-five c.c. of distilled water. The jars were then covered and vigorously shaken at short intervals, after which they were allowed to stand until clear. Aliquots were then evaporated on the water bath, and the determinations made in the usual way. Except in table II the data given represent the average of duplicate determinations.

## Nitrification of Pyridine, Quinoline, Quanidine Carbonate, Etc.

To determine whether the compounds used in this work were nitrified in the soil to an appreciable extent, and to determine the effect of lime on the nitrification, the methods described above were used. The period of incubation varied with the soil used, from three and one half to five months. Three different soils were used. Soil No. 1 was obtained from a plot on the Experiment Station Farm which had received annually a moderate application of ammonium sulphate, and is quite acid. The experiment on this soil ran from June 15 to September 21, 1916. Soil No. 2 was obtained from what is known as the 'Cullers Rotation Field," about one mile south of Auburn. This soil, classed as Norfolk sandy loam by the Bureau of Soils, is slightly acid. The test ran from June 16 to September 16, 1916. Soil No. 3 came from what is known as the "square acre" on the Experiment Station Farm. It is moderately acid. This experiment ran from Feb. 18 to June 21, 1915. The treatments given, and the amounts of nitrates found in the soils, in both limed and unlimed conditions, are shown in table I.

Table I.—*Nitrification of Dried Blood, Pyridine, Quinoline, Etc. Nitrates Expressed as p. p. m. of Dry Soil.*

| Treatment | Soil No. 1 | | Soil No. 2 | | Soil No. 3 | |
|---|---|---|---|---|---|---|
| | | With lime | | With lime | | With lime |
| Dry checks | None | ------ | 1.3 | ------ | 1.3 | ------ |
| Dist. water | 56.0 | 145.0 | 58.7 | 177.5 | 58.3 | 145.0 |
| Dried blood | 145.0 | 480.0 | 235.0 | 390.0 | 260.0 | 460.0 |
| Pyridine | 192.5 | 240.0 | 185.0 | 245.0 | 145.0 | 185.0 |
| Quinoline | 152.5 | 20.0 | 55.0 | Trace | 215.0 | 180.0 |
| Piperidine | 155.0 | 290.0 | 165.0 | 325.0 | 268.0 | 400.0 |
| Guanidine carbonate | 200.0 | 2.0 | ------ | ------ | 140.0 | 6.8 |
| Nucleic acid | ------ | ------ | ------ | ------ | 245.0 | 530.0 |
| Alloxan | ------ | ------ | ------ | ------ | 275.0 | 540.0 |
| Asparagine | ------ | ------ | ------ | ------ | 275.0 | 540.0 |
| Napthylamine | ------ | ------ | ------ | ------ | 1.6 | 2.2 |

A study of the data presented in table I. shows clearly that, of all the compounds used, and in each of the three different soils, naphthylamine and quinoline in soil No. 2, were the only substances which were not nitrified. Of the three soils used, No. 1 is most acid, and No.2 is least acid. Quinoline was apparently not nitrified in the least acid soil, but in the soils which were moderately or strongly acid, this basic compound was nitrified to quite an appreciable degree. Further, the addition of lime to the least acid soil inhibited nitrification, but only reduced the process in the more acid soil. In both limed and unlimed conditions, pyridine was nitrified in each of the soils used. But the effect of lime on the nitrification of this basic compound is not nearly so great as in the case of dried blood or the other non-basic materials. The fact that so stable a compound as pyridine, which is unaffected by boiling nitric acid, or chromic acid, is nitrified in these soils, illustrates in a very definite way the enormously destructive chemical and biochemical action that may take place in soils. Piperidine was nitrified in each of the soils, the addition of lime increasing the amount of nitrification in each case; this too, in spite of the fact that the compound is of a strong basic character. In the unlimed acid soils, guanidine carbonate, another basic compound, was readily nitrified; in the limed series, however, nitrification was inhibited. Nucleic acid, alloxan, asparagine and naphthylamine were used in but one soil. Of these, only naphthylamine was not nitrified. As in the case of dried blood, the decomposition of these non-basic substances was greatly increased by the addition of lime.

## The Effects of Varying Amounts of Nitrogenous Compounds on Nitrification

The effect of concentration of the nitrogenous compounds on nitrification was also studied. The soil used in this case was a poor, sandy soil which had been left undisturbed in the green house for several months, and had accumulated quite a large amount of nitrates. Each tumbler contained ninety-seven grams of this poor sand, one gram of fertile soil and two grams of lime. The period of incubation lasted from July 11th to September 8th, 1915.

TABLE II.—*Effect of Varying Amounts of Nitrogenous Compounds on the Rate of Nitrification.*

| Treatment | NO3 in p. p. m. of air dry soil from | | | |
|---|---|---|---|---|
| | 0 | 0.5 gram | 0.25 gram | 0.1 gram |
| Dry checks | 137 | | | |
| Distilled water | 231 | | | |
| Dried blood | | 1225 | 850 | 525 |
| Asparagine | | 175 | 550 | 486 |
| Napthylamine | | 187 | lost | 175 |
| Alloxan | | 187 | 200 | 525 |
| Nucleic acid | | 360 | 237 | 625 |
| Quinoline | | 175 | 200 | 200 |
| Pyridine | | 200 | 212 | 225 |

With the exception of dried blood and naphthylamine, more nitrates were formed from ten tenth gram treatments than from the half gram treatments. The heavier applications of asparagine, alloxan, and nucleic acid exerted a decided inhibitory effect on nitrification. Compared with the distilled water check, pyridine, and quinoline slightly retarded nitrification. It is probable that this experiment was of too short duration for the last named compounds to be decomposed in the very poor soil used. Or, the two grams of lime per tumbler may have retarded their nitrification, since in other experiments lime has been found to be inhibitory to the decomposition of quinoline, and of but little benefit to the decomposition of pyridine.

THE EFFECT OF PARTIAL STERILIZATION OF SOIL ON NITRIFICATION OF PYRIDINE, QUINOLINE, ETC.

In view of the fact that various investigators have reported that treatment of the soil with carbon disulphide destroys the nitrifying organism, it was thought of interest to study the effect of "partial sterilization" on the nitrification of these compounds. Carbon disulphide was added to a number of tumblers in sufficient quantity to moisten about one half of the 100 gram samples of soil used. After standing covered for about 24 hours, the soil in the tumblers was exposed to the air and allowed to stand for about ten days so as to rid the soil of the antiseptic. One series received no lime, a second series was limed, and a third series was limed and reinoculated with an infusion from the untreated soil. During the time that this experiment was in progress, no special attempt was made to avoid contamination of the soil treated with the antiseptic. However, distilled water was used throughout, and each tumbler was kept covered in a dark closet. The soil used came from a plot on the Experiment Station Farm which was very acid, due largely to the application of sulphate of ammonia in the field treatment. The test ran from June 15 to September 17, 1916.

TABLE III.—*The Effect of Partial Sterilization of Soil on the Nitrification of Pyridine, Quinoline, Etc.*

| Treatment | $NO_3$ in p. p. m. of soil treated with | | |
|---|---|---|---|
| | 0 | Lime | Reinoculated and lime |
| Dry checks | 0 | ---------- | ---------- |
| Distilled water | 56 | 145 | ---------- |
| Distilled water and $CS_2$ | trace | 112 | ---------- |
| Dried blood | 145 | 480 | ---------- |
| Dried blood and $CS_2$ | trace | 233 | 380 |
| Pyridine | 192 | 240 | ---------- |
| Pyridine and $CS_2$ | 58 | 305 | 385 |
| Quinoline | 152 | 20 | ---------- |
| Quinoline and $CS_2$ | trace | trace | 21 |
| Piperidine | 155 | 290 | ---------- |
| Piperidine and $CS_2$ | 19 | 381 | 427* |

* In recording the data, fractions of a part per million were frequently omitted if the nitrate content was great.

The results which are tabulated in table III. show that nitrifying organisms gained entrance to the tumblers, or that the carbon disulphide treatment failed to destroy all of the nitrifying bacteria. From unpublished data obtained by the writer, he believes that the presence of nitrifying organisms in the soil treated with carbon disulphide was not due to contamination, but to the fact that the organisms were not killed by the antiseptic.

Carbon disulphide inhibited nitrification in the untreated soil and in the soil treated with dried blood or quinoline, and seriously reduced the process in soil treated with pyridine or piperidine. In the presence of lime, the antiseptic reduced nitrification in the untreated soil and in the soil treated with dried blood or with quinoline. On the other hand, with carbon disulphide and lime, more nitrates were formed from pyridine and from piperidine than were obtained from these compounds alone. The effect of reinoculation was to increase in all cases the amounts of nitrates recovered.

## The Effect of Non-Nitrogenous Substances Toxic to Plants in Water Cultures, on the Nitrification of Dried Blood, Pyridine, Etc.

Davidson (3) has shown that the addition of vanillin or cumarin to soil which had been treated with lime, sodium nitrate, or potassium chloride, decreased nitrification; while, in the presence of disodium phosphate, there was greater nitrification in the vanillin and cumarin treated soils than in the controls. He suggests that the action of vanillin and cumarin on nitrification may be analogous to the behavior of soluble organic matter in general.

The writer studied the effect of vanillin, cumarin, pyrogallol and salicylic aldehyde on the nitrification of dried blood, pyridine, quinoline, piperidine and guanidine carbonate, in both limed and unlimed conditions. The methods employed were the same as indicated above.

Two soils were used, one of which, a Norfolk sandy loam, was obtained from the Cullers rotation plots; and the other was obtained from the very acid plot on the Experiment Station Farm.

In the slightly acid Norfolk sandy loam soil, as can be seen from table IV. dried blood, pyridine and piperidine produced notable increases in the nitrate content of the soil. From quinoline, however, not quite as much nitrates were produced as from the soil treated with water only. The addition of lime to quinoline inhibited nitrification; but, with dried blood, pyridine, and piperidine, lime increased nitrification.

Pyrogallol alone retarded nitrification of dried blood and pyridine, but slightly increased nitrate formation from quinoline and piperidine. Comparing the pyrogallol-lime series with the nothing-lime series, less nitrates were obtained in the presence of pyrogallol than where it was absent. It is apparent, however, that the inhibitory action of pyrogallol is quite largely overcome by lime.

Vanillin alone practically inhibited nitrification of pyridine and quinoline, seriously reduced the process with dried blood, but had very little effect on the nitrification of piperidine. Lime reduced to a marked degree the toxic action of vanillin toward the nitrification of pyridine and dried blood. In no instance was the nitrate content of the vanillin-lime series as high as in the nothing-lime series, showing that lime failed to completely overcome the bad effect of the vanillin.

TABLE IV.—*The Effect of Non-Nitrogenous Substances on the Nitrification of Dried Blood, Pyridine, Etc., in Norfolk Sandy Loam Soil. Nitrates Expressed as p. p. m. of Dry Soil.*

| Nitrogenous compounds added | Nothing | | Pyrogallol | | Vanillin | | Cumarin | | Salicylic aldehyde | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | With lime | | With lime | | With lime | | With lime | | With lime |
| Dry check | 1.3 | | | | | | | | | |
| Dist. water | 58.7 | 177.5 | | | | | | | | |
| Dried blood | 235.0 | 390.0 | 101.0 | 300.0 | 122.0 | 275.0 | 210.0 | 352.0 | trace | trace |
| Pyridine | 185.0 | 245.0 | 150.0 | 197.0 | 4.8 | 200.0 | 112.0 | 56.0 | trace | trace |
| Quinoline | 55.0 | trace | 67.0 | trace | trace | trace | trace | trace | trace | trace |
| Piperidine | 165.0 | 325.0 | 205.0 | 260.0 | 160.0 | 285.0 | 152.0 | 297.0 | trace | trace |

Nitrification of dried blood and piperidine was but slightly reduced by cumarin alone. However, this compound materially reduced nitrification of pyridine, and inhibited the process with quinoline. Lime increased the toxicity of cumarin to nitrification of pyridine, but greatly reduced it to the decomposition of dried blood and piperidine. No nitrates were formed from quinoline with or without lime. In those instances where lime reduced toxicity of cumarin, comparison with the nothing-lime series shows that the toxicity was not entirely overcome.

Salicylic aldehyde completely inhibited nitrification in all cases.

In all cases, in the very acid soil, the data for which are given in table V. the various nitrogenous compounds gave rise to more nitrates than were obtained from the distilled water check. Attention is called to the fact that in this very acid soil, quinoline is nitrified, while in the slightly acid Norfolk sandy loam, it is not nitrified. Lime increased nitrification in each case except in the quinoline and quanidine carbonate treated soil, in which instances lime markedly reduced nitrification. From the data given, it is evident that lime reduces or completely inhibits nitrification of quinoline. On the other hand, this basic compound is apparently most readily nitrified in the soil having the highest acidity.

TABLE V.—*The Effect of Non-Nitrogenous Substances on the Nitrification of Dried Blood, Pyridine, Etc., in Acid Soil from the Experiment Station Farm. Nitrates Expressed as p. p. m. of Dry Soil.*

| Nitrogenous compounds added | Nothing | | Pyrogallol | | Vanillin | | Cumarin | | Salicylic aldehyde | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | With lime | | With lime | | With lime | | With lime | | With lim |
| Dry check | 1.3 | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ |
| Dist. water | 58.7 | 177.5 | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ |
| Dried blood | 145.0 | 480.0 | 47.0 | 180.0 | 92.0 | 305.0 | 97.0 | 365.0 | trace | trace |
| Pyridine | 192.0 | 240.0 | 107.0 | 275.0 | 130.0 | 345.0 | 195.0 | 240.0 | trace | trace |
| Quinoline | 152.0 | 20.0 | 90.0 | trace | trace | trace | 152.0 | trace | trace | trace |
| Piperidine | 155.0 | 290.0 | 125.0 | 360.0 | 130.0 | 435.0 | 132.0 | 245.0 | 22.3 | 38.0 |
| Guanidine carbonate | 200.0 | 2.0 | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ |

Pyrogallol alone materially retarded the nitrification of dried blood, pyridine and quinoline, and slightly retarded nitrification of piperidine. Compared with the nothing-lime series, pyrogallol with lime increased the nitrification of dried blood, pyridine and piperidine, but inhibited the process with quinoline.

In the presence of vanillin alone, each of the compounds was nitrified less than when vanillin was absent. On the other hand, two of the compounds were nitrified more with vanillin and lime than with lime alone. Just why the combined action of pyragallol and lime, or vanillin and lime, should favor nitrification more than does the lime alone, is not clear.

By the addition of cumarin, nitrification of dried blood and piperidine was reduced considerably below that found in the nothing series. Pyridine and quinoline, however, were nitrified as well with cumarin present as without cumarin. In the presence of lime, the inhibitory effect of cumarin on the nitrification of dried blood and piperidine was materially reduced; while there was no effect from lime in the pyridine-cumarin treated soil. Lime in addition to cumarin completely inhibited nitrification of quinoline.

Salicylic aldehyde inhibited nitrification in all instances except with piperidine, in the presence of which compound a small amount of nitrate was found in the unlimed series. Salicylic aldehyde persists in soils longer, as indicated by smell, than any other compound used in the experiments here reported.

## The Effect of Combinations of Non-Nitrogenous Compounds Toxic to Plants in Solution Cultures, on the Nitrification of Dried Blood, Pyridine, Etc.

A study of the effect of one, or the combined effect of two, or three, non-nitrogenous compounds on nitrification was made in 1915, using Norfolk sandy loam soil similar to that used in other tests reported on the previous pages. The data obtained are presented in table VI.

Table VI.—*The Effect of Combinations of Non-Nitrogenous Compounds on the Nitrification of Dried Blood, Pyridine, Etc. Nitrates Expressed as p. p. m. of Dry Soil.*

| Nitrogenous compounds added | Nothing | | Vanillin | | Vanillin and cumarin | | Vanillin, cumarin and dihydroxystearic acid | |
|---|---|---|---|---|---|---|---|---|
| | | With lime | | With lime | | With lime | | With lime |
| Dist. water | 135.0 | ------ | ------ | ------ | ------ | ------ | ------ | ------ |
| Dried blood | 290.0 | 540.0 | 180.0 | 490.0 | trace | 71.0 | 9.0 | 100.0 |
| Pyridine | 215 | ------ | 85.0 | ------ | trace | ------ | trace | 55.0 |
| Quinoline | 185.0 | ------ | 7.0 | ------ | trace | ------ | trace | 37.0 |
| Piperidine | 265.0 | ------ | 280.0 | ------ | trace | ------ | trace | 112.0 |
| Guanidine Carbonate | 445.0 | ------ | 19.0 | ------ | trace | ------ | trace | trace |

In the soil otherwise untreated each of the compounds was nitrified, the highest nitrate content being found in soil treated with guanidine carbonate, and the lowest, in soil treated with quinoline.

By the addition of vanillin, nitrification of all compounds was seriously reduced, with the exception of piperidine, from which more nitrates were formed with vanillin than without it.

The combined action of vanillin and cumarin was to inhibit completely the nitrification of all compounds. Dried blood was slightly nitrified in the presence of these two non-nitrogenous compounds, where lime was added; unfortunately, lime was not used in connection with the other nitrogenous substances.

In the presence of vanillin, cumarin, and dihydroxystearic acid, nitrification was inhibited in all cases except in the soil treated with dried blood, in which there was found a very small amount of nitrates. In the limed series, however, a varying quantity of nitrates was recovered from each treatment except guanidine carbonate; the amount recovered from the soil treated with dried blood and piperidine averaged 100 and 112 parts per million of soil, respectively. The great corrective effect that lime may exert in soil is very strikingly shown by the results set forth above.

### Influence of Lime, Carbon Black, and Pyrogallol on Nitrification of Dried Blood in Presence of Vanillin Cumarin, and Dihydroxystearic Acid.

It has been shown that the toxicity of soil extracts may be partially or even completely removed by the use of such compounds as carbon black, pyrogallol or lime. It was thought probable, therefore, that these compounds might have a beneficial effect on nitrification in soil to which toxic compounds were added. To test this, a number of tumblers were prepared in the usual way, and treated as shown in table VII.

From each of the comparisons which may be made with this data, it is seen that pyrogallol exhibited an inhibitory effect on nitrification.

Lime had a beneficial effect on nitrification under each of the conditions in which it was used. This result was to be expected from the numerous instances given in which the action of lime was shown to be helpful.

Table VII.—*The Effect of Lime, Pyrogallol and Carbon Black on Nitrification of Dried Blood in Presence of Vanillin, Cumarin, and Dihydroxystearic Acid. Nitrates Expressed as p. p.m. of Dry Soil.*

| Corrective materials added | Dried blood | Dried blood and vanillin | Dried blood, vanillin, cumarin | Dried blood vanillin, cumarin, Dihydroxy-stearic acid |
|---|---|---|---|---|
| None | 290.0 | 180.0 | trace | 9.0 |
| Lime | 540.0 | 490.0 | 71.0 | 100.0 |
| Carbon black | ---------- | 190.0 | 140.0 | 105.0 |
| Pyrogallol | ---------- | 55.0 | trace | trace |
| Lime and carbon black | ---------- | ---------- | ---------- | 370.0 |
| Carbon black and pyrogallol | ---------- | ---------- | ---------- | 47.0 |
| Lime, carbon black and pyrogallol | ---------- | ---------- | ---------- | 182.0 |

The results obtained with carbon black are very interesting. In no case was there an apparent harmful effect, and in several instances there was marked benefit to the process of nitrate formation. Carbon black materially reduced the toxic effect of vanillin and cumarin; and of vanillin, cumarin and dihydroxystearic acid. Indeed, the combined effect of lime and carbon black was to overcome quite largely the toxicity of cumarin, vanillin, and dihydroxystearic acid combined.

## Discussion

The evidence here brought together would seem to show that, under the conditions of these experiments, even extremely stable nitrogenous compounds may be decomposed in soils. The recent results obtained by Robbins (8) indicate that such decomposition may be due, wholly or in part, to the action of soil organisms. In view of such decomposition, it is very doubtful if these or similar substances would accumulate in soils, under similar conditions, in sufficient quantities to become harmful to growing plants.

A very interesting point developing from this work is the indication that *certain basic compounds, as quinoline or guanidine carbonate, are nitrified best in acid soils, and that liming of acid soils prevents the decomposition of such substances.* Indeed, the addition of lime may not only prevent the nitrification of quinoline and guanidine carbonate, but the combined action of lime and these basic substances may also suppress the nitrification of organic compounds normally occurring in soils. Evidence to support this view may be found in tables I., IV. andV. A possible explanation may be that the organisms which have the ability to decompose these compounds function only in acid soils. Or, it may be that the first stages of decomposition are effected by organisms other than bacteria, and that these are not active in the limed soils. Further, the basic compounds may form salts in the acid soils, which salts are more readily nitrified than the original compounds. If this last view be correct, the suppression of nitrification in the limed soils may be due to the prevention by lime of salt formation between quinoline and guanidine carbonate, and acid bodies existing in soils. Not all of the basic compounds, however, were affected alike by liming, since *pyridine was decomposed in the limed soils, and the decompo-*

*sition of piperidine was considerably increased by liming.* From the foregoing it might be suggested that a different organism, or group of organisms, should be involved in the decomposition of such compounds.

Another interesting point which deserves mention is the non-toxic action of vanillin toward the decomposition of piperidine. In vanillin treated soils nitrification of dried blood and pyridine was seriously reduced, and of quinoline, practically inhibited. But the decomposition of piperidine was about as great in the presence of vanillin as in the untreated soils. There are several possible explanations of these differences. The decomposition of piperidine may be caused by an organism, or group of organisms, which are unaffected by vanillin; while the nitrification of quinoline, etc., may be due to an organism or group of organisms to which vanillin is toxic. Again, vanillin may be toxic to the nitrification of piperidine, quinoline and similar substances. But in presence of piperidine, certain organisms destroy the vanillin, thereby permitting piperidine decomposition to proceed; while in the presence of quinoline the action of the vanillin destroying organism or organisms is inhibited. In other owrds, there might be a mutual inhibitation of the organisms requisite to quinoline and vanillin decomposition.

It is rather generally accepted that liming an acid soil increases nitrification, and this view seems to be correct for a large number of compounds. However, quinoline and quanidine carbonate are two exceptions to be noted among the compounds used in this work. And it is conceivable that other basic nitrogenous compounds may be nitrified best in acid soils. Again, the fact that a substance inhibits the nitrification of one compound is not proof that the compound inhibits nitrification in general. For example, vanillin reduces or even inhibits the nitrification of quinoline, while the nitrification of piperidine seems to be but little affected by the addition of vanillin. Likewise, cumarin may exert a decided inhibitory effect on the nitrification of one compound and but little on another. Further, the inhibitory action of a compound toward nitrification may be quite different in different soils. In view of the results here given, a general rule in regard to nitrification would appear to be impossible.

In most instances lime greatly reduced the toxicity of pyrogallol, vanillin and cumarin, toward nitrification. The beneficial results obtained with lime may be due to the neutralization of soil acidity, thereby increasing bacterial activity, or the lime may react, directly or indirectly, with these compounds, the resultant products being less injurious than the original.

In several instances, greater nitrification was found in the lime-pyrogallol series and the lime-vanillin series than in the lime series alone. It may be possible to explain these rather peculiar results, in the light of recent work by Doryland and by Robbins. The results obtained by Doryland (4) tend to show that while a large amount of energy-producing material may give rise to such large numbers of bacteria that the ammonia produced from a nitrifiable substance may be entirely consumed by the bacteria, a small amount of energy-producing material may actually increase ammonia accumulation. The explanation offered is that the small amount of energy-producing material induces large numbers of bacteria in the medium. When this small amount of energy-producing material is exhausted the nitrogenous compounds remaining are attacked as a source of energy, causing rapid ammonification and ammonia accumulation. Robbins (8) has shown that the addition of cumarin or vanillin to soil enormously increases the number of bacteria present. He has shown, further, that certain bacteria are able to use these compounds as sources of energy. It may be possible, therefore, to explain the increased nitrification in the presence of vanillin as being due to the increased numbers of bacteria induced by the small amounts of vanillin added. The vanillin in this case producing effects similar to those found by Doryland, where small amounts of energy-producing material like dextrose caused increased ammonia accumulation.

## SUMMARY

(1) With the exception of naphthylamine, each of the compounds used in this study was nitrified in soil.

(2) At the concentration used, naphthylamine inhibited nitrification in both limed and unlimed soil.

(3) Quinoline was nitrified most readily in soil having the highest lime requirement. Lime retarded or even inhibited nitrification of quinoline.

(4) Lime practically inhibited nitrification of guanidine carbonate. Nitrification of dried blood, piperidine, nucleic acid, alloxan, and asparagine, was greatly increased by lime.

(5) Heavy applications of certain nitrogenous compounds may retard nitrification.

(6) Liming a soil which had been partially sterilized with carbon disulphide greatly increased its power of nitrification. A still further increase was obtained by reinoculation of the soil after partial sterilization.

(7) Vanillin proved to be non-toxic toward nitrification of piperidine, moderately toxic toward nitrification of dried blood and pyridine and inhibitory toward nitrification of quinoline.

(8) Lime counteracted the toxicity of vanillin to a very large degree.

(9) The effect of cumarin on nitrification was quite variable. In some instances it exerted an inhibitory effect; in others, none.

(10) In most cases where cumarin exerted an inhibitory effect, lime greatly reduced the amount of inhibition.

(11) Pyrogallol retarded nitrification of all compounds, except quinoline and piperidine, in one soil.

(12) Lime reduced the injurious effect of pyrogallol in all cases, except in the quinoline treated soil.

(13) Salicylic aldehyde completely inhibited nitrification of all compounds, except piperidine, in one soil.

(14) Carbon black apparently overcomes a part of the bad effect of certain non-nitrogenous compounds on the process of nitrification.

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