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THE DECOMPOSITION OF TOXINS BY SOIL ORGANISMS

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I. INTRODUCTION*

In most of the published literature scant consideration has been given to the possibilities of eliminating the evil effects on plants of the organic toxins through the agency of soil organisms. Tilth, reaction, aeration, and fertilization have each received abundant and detailed consideration as means of correcting these effects, while the microorganisms of the soil have been considered as means of converting dead plants and animals into humus, or else as means of rendering organic matter available for plant metabolism. Except for the work of Robbins (13, 14, 15) the recognition of toxin decomposing organisms has been limited to the mention of "biological agencies" capable of improving the soil.

It has seemed desirable to determine some of the conditions for the growth of toxin decomposing organisms, to ascertain which toxins may be decomposed, and to learn the distribution of these organisms. Investigations along these lines are reported in the following pages.

The background of these investigations has been discussed fully by Schreiner and Reed (17), Schreiner and Shorey (21), Hopkins (8), Skinner (24), and Robbins (14). The more recent papers will be mentioned in connection with particular toxins.

The materials employed in these investigations consisted of chemically pure inorganic salts, the highest quality toxins, and soils collected under aseptic conditions wherever possible.

*NOTE—The writer wishes to acknowledge the assistance of A. B. Massey, G. R. Johnstone and Dewey Stewart in certain phases of these investigations.

II. DECOMPOSITION OF NON-NITROGENOUS TOXINS BY SOIL ORGANISMS

1. NUTRIENT SOLUTIONS

In the search for a transparent medium which would yield adequate and consistent growth of bacteria and molds under favorable conditions the well known nutrient solutions of Pfeffer, Crone, Sachs, Birner, Lucanus, and Knop were tested with the organisms of several soils as well as the nutrient solution used by Robbins (14). The results show that of those tested, Robbins' solution is the most suitable substratum for the organisms used.

While the results mentioned above show that Robbins' solution (lacking calcium salts) was most satisfactory for the organisms of the several soils tested, the fact remains that calcium is necessary for the growth and vigor of many plants, even though magnesium may meet the needs in some cases. Moreover, the other preliminary experiments indicate that the growth of some toxin decomposing organisms may be favored by the presence of calcium sulphate; again, when calcium sulphate and potassium hydrogen phosphate in solution are heated together a precipitate is formed, which makes observation of the growth of bacteria difficult. Since all of the calcium and phosphorus might be contained in the precipitate and thus scarcely available for microorganisms, it was thought that the use of a soluble phosphate of calcium such as monobasic calcium phosphate might eliminate these difficulties, and at the same time permit the presence of a soluble calcium salt in the medium.

In order to determine more definitely the utility and the most available form of calcium for the microorganisms under investigation, three experiments were performed in which Robbins' solution with one gram of dextrose per liter added was used as the basis.

In the first experiment flasks, each containing about 50 cc. of the modified Robbins' solution (lacking the calcium salt, but containing one gram of dextrose per liter), were sterilized and then inoculated with a certain soil. This was repeated for 24 other selected soils. The cultures were incubated at room temperature for 12 days when examination revealed the results tabulated in Table I under "No Calcium Salt."

In the second experiment 0.2 gram of monobasic calcium phosphate $Ca(H_2PO_4)_2$ was added to each liter of modified Robbins' solution, and cultures made as indicated above. Observation after incubating 12 days revealed the results tabulated under "Monobasic Calcium Phosphate."

In the hope of securing more abundant growth of bacteria, cultures were prepared for the third experiment in the usual manner except that 0.2 gram calcium sulphate was added to

each liter of modified Robbins' solution. These cultures were incubated 7 days at room temperature, and when examined for growth of bacteria and molds the results tabulated under "Calcium Sulphate" were obtained.

TABLE I.—*Calcium Salts and the Growth of Microorganisms*

Soil	No Calcium Salt		Monobasic Calcium Phosphate		Calcium Sulphate	
	Bacteria	Molds	Bacteria	Molds	Bacteria	Molds
7 -----	no	yes	no	yes	yes	no
8 -----	no	yes	no	yes	yes	yes
9 -----	no	yes	no	yes	yes	no
10 -----	no	yes	no	yes	yes	yes
12 -----	no	yes	no	yes	yes	yes
14 -----	yes	yes	yes	no	yes	no
16 -----	no	yes	no	no	no	yes
17 -----	no	yes	no	yes	no	yes
19 -----	yes	yes	yes	yes	yes	yes
20 -----	yes	yes	no	yes	yes	yes
21 -----	no	yes	no	yes	no	yes
25 -----	no	yes	no	yes	yes	yes
26 -----	no	yes	no	yes	no	yes
27 -----	no	yes	no	yes	yes	yes
28 -----	yes	yes	no	yes	yes	no
29 -----	no	yes	no	yes	no	yes
30 -----	no	yes	no	yes	no	yes
31 -----	no	yes	no	yes	yes	yes
32 -----	no	yes	no	yes	yes	yes
33 -----	yes	yes	no	yes	yes	yes
34 -----	no	yes	no	yes	yes	yes
35 -----	no	yes	no	yes	yes	no
36 -----	no	yes	no	yes	no	yes
129 -----	no	yes	yes	yes	yes	yes
130 -----	no	yes	no	yes	yes	yes

From Table I it appears that but five of the 25 soils contained bacteria which multiply in the calcium-salt-free medium while each of the same soils contained molds which grow in this medium. It appears also that the bacteria of 17 and the molds of 22 of the 25 soils were able to grow when calcium sulphate was added to Robbins' solution.

These results indicate that for soil bacteria a medium containing calcium as a sulphate is superior both to a medium lacking calcium and to one containing monobasic calcium phosphate. Calcium either as sulphate or monobasic phosphate does not seem to materially promote the growth of molds.

Calcium Acid Phosphate and the Decomposition of Toxins

Before attempts to secure a transparent nutrient solution were abandoned, cultures were made in which the nutrient solution contained monobasic calcium phosphate as the source

of calcium, and a toxin as the source of carbon. Such toxins as cinnamic acid, hydroquinone, and quinone were used. In nearly all cases the growth of bacteria was as meagre as, and usually the mold growth was less than, in cultures where dextrose was the source of carbon as detailed above. The other phosphates tested were no more satisfactory. They either yielded precipitates, or else they inhibited growth. When monobasic calcium phosphate was omitted, or replaced with calcium sulphate satisfactory growth of the toxin decomposing bacteria and molds occurred.

2. THE REACTION OF THE SUBSTRATUM

In testing for the best form of calcium for use in cultures of soil organisms it was noticed that the molds grew in more of the cultures than did bacteria. This suggested that the medium was too acid for optimum growth of bacteria. Having approximated a medium of suitable constituents it seemed desirable to determine more definitely the best reactions for organisms which decompose toxins such as resorcinol, cumarin, cinnamic acid and vanillin. For these experiments organisms in pure culture known to be able to grow and thrive on toxins (and in some cases to decompose them) were used in media of different reactions. The incubation period was two weeks, which gave ample time for the organisms at room temperature to decompose the small amount of toxin present.

Reaction for Resorcinol Decomposing Organisms

To determine the best reaction of substratum for organisms which decompose resorcinol, Robbins' solution to which was added 0.5 gram of resorcinol per liter, was prepared according to the formula given below and divided into three lots.

One lot was left in natural condition with a reaction of 0.5% acid normal to phenolphthalein, equivalent to the acidity of a liter of solution to which 5 cc. of normal hydrochloric acid had been added. Another lot was made neutral by adding 5 cc. of normal sodium hydroxide per liter. Another was made 1.0% acid normal by adding 5 cc. of normal hydrochloric acid per liter. These solutions were distributed to small Erlenmeyer flasks with 50 cc. to each flask and sterilized. The medium in the flasks was inoculated in duplicate with a pure culture of mold or bacteria from certain soils respectively and set to incubate.

Medium	
Dipotassium hydrogen phosphate	0.50 gram
Sodium nitrate	0.50 gram
Ammonium chloride	0.50 gram
Magnesium sulphate	0.20 gram
Potassium chloride	0.20 gram
Ferric chloride	trace
Resorcinol	0.50 gram
Distilled water	1000 cc.

TABLE II.—*Reaction for Resorcinol Decomposing Organisms*

	Natural Reaction or 0.5% acid normal		Natural Reaction plus 5 cc. n/l NaOH per liter		Natural Reaction plus 5 cc. n/l HCL per liter	
Organism	Growth	Toxin	Growth	Toxin	Growth	Toxin
5B -----	yes	yes	no	yes	yes	yes
5M ----	yes	no	yes	no	yes	no
15B -----	yes	no	yes	yes	yes	yes
15M -----	yes	no	yes	no	yes	no
20B -----	yes	yes	yes	yes	yes	yes
20M -----	yes	no	yes	no	yes	no
26B -----	no	yes	yes	yes	yes	no
26M -----	yes	no	no	yes	no	yes
Control -	no	yes	no	yes	no	yes

From the above results it appears that resorcinol is not readily decomposed by bacteria, though it is decomposed by molds where the reaction ranges from 0.0% to 1.0% acid normal to phenolphthalein. Most abundant decomposition occurred where the reaction was 0.5% acid normal to phenolphthalein.

Reaction for Organisms Which Decompose Cumarin

The inocula consisted of pure cultures of organisms which had been found able to decompose the toxin under ordinary conditions. The inoculation, incubation, and conditions were as usual except for the variation of the reaction (0.4, 0.9, and 1.4%) as indicated below. The substratum consisted of Robbins' solution modified by the addition of 0.5 gram of calcium sulphate to which 0.5 gram of cumarin had been added.

TABLE III.—*Reaction for Cumarin Decomposing Organisms*

	Natural Reaction plus 5 cc. normal NaOH per liter		Reaction Natural 0.9%		Natural Reaction plus 5 cc. normal HCL per liter	
Organism	Growth	Toxin	Growth	Toxin	Growth	Toxin
5a2b ---	yes	no	yes	no	no	yes
21a1b ---	yes	no	yes	no	no	yes
21a2b ---	yes	no	yes	no	no	yes
22a1b ---	yes	no	yes	yes	no	yes

From these results it appears that the best reaction tested for the decomposition of cumarin by soil organisms, is 0.4% acid normal to phenolphthalein.

The Reaction for Cinnamic Acid Decomposing Organisms

This test was carried out in the same manner as that for cumarin organisms, except for the substitution of cinnamic acid for cumarin and slight variations in the reactions as shown below.

TABLE IV.—*Reaction for Cinnamic Acid Decomposing Organisms*

	Natural Reaction plus 5 cc. normal NaOH per liter		Natural Reaction 0.7%		Natural Reaction plus 5 cc. HCl per liter	
Organism	Growth	Toxin	Growth	Toxin	Growth	Toxin
5aB ----	yes	yes	no	yes	no	yes
15am ---	yes	no	no	yes	no	yes
20a2m --	yes	no	no	yes	no	yes
21a2B ---	yes	no	no	yes	no	yes
22a2B ---	yes	no	yes	no	no	yes
26am ---	yes	no	no	yes	no	yes

From the results reported above it appears evident that neither bacteria nor molds can grow in media containing cinnamic acid if the acid reaction exceeds 1.0%. The best growth occurred in the solution with a reaction of 0.2% acid normal to phenolphthalein.

The Reaction for Soil Organisms Which Decompose Vanillin

This test was carried out in the same manner as the above.

TABLE V.—*Reaction for Vanillin Decomposing Organisms*

	Natural Reaction plus 5 cc. NaOH per liter		Natural Reaction 0.6%		Natural Reaction plus 5 cc. normal HCl per liter	
Organism	Growth	Toxin	Growth	Toxin	Growth	Toxin
5a2B --	yes	no	yes	no	no	yes
5a1B --	yes	no	yes	no	no	yes
21a B --	yes	no	yes	no	no	yes
22a1B --	yes	no	yes	no	no	yes
43a B --	yes	no	yes	no	no	yes
130 1B --	yes	no	yes	no	no	yes

These results indicate that the optimum reaction for vanillin decomposing organisms is well below 1.1%. This deduction is supported by other experiments in which complete decomposition of the toxin occurred almost simultaneously whether

the reaction was .1%, .2%, .3%, or .4% acid normal to phenolphthalein, though decomposition occurred a little sooner in a medium having a reaction of .4% acid normal to phenolphthalein. (This is equivalent to a pH value of 6.9).

These results are somewhat at variance with those obtained by Robbins and Massey (16) with vanillin organisms. They found best decomposition of vanillin in a solution to which they had added 1 cc. of tenth normal sodium hydroxide per 100 cc. of a medium which was neutral to phenolphthalein. A solution prepared according to their formula had a pH value of 4.80. It may be added that details of preparation of the medium, such as time of heating as well as buffer effects, have considerable influence on the reaction of the medium.

From the results with resorcinol, cumarin, cinnamic acid, and vanillin it is apparent that the best reaction of the substratum for toxin decomposing organisms is often the natural reaction, and lies between 0.1% and 0.5% acid normal to phenolphthalein.

3. VANILLIN

This substance, m-methoxy-p-oxybenzaldehyde, which is produced by splitting a certain glucoside, has been the subject of much investigation. In 1907 Schreiner, Reed, and Skinner (20) reported it as injurious to wheat seedlings in solution cultures. Shorey (23) reported the isolation of vanillin from a Florida soil in 1914. Fraps (3) in 1915 found it injurious to the growth of one of eight crops if applied at time of planting. The next year Funchess (4) showed that the addition of large amounts of vanillin depresses the yield when oats are planted immediately after vanillin is applied, and that fertilizers do not prevent its bad effects. A year later Robbins (15) demonstrated the decomposition of vanillin by the organisms of certain soils. The writer (6) has shown that the organisms which decompose vanillin are not able to decompose other toxins like cumarin and resorcinol. Besides the optimum reaction for vanillin decomposing organisms and the best nutrient solution already reported in this bulletin, there remains the consideration of the occurrence and distribution of vanillin decomposing organisms.

Occurrence of Vanillin Decomposing Organisms

To get some idea of the occurrence of vanillin decomposing organisms Robbins' solution, having the natural reaction to which was added 0.5 gram of vanillin per liter, was placed in Erlenmeyer flasks, 50 cc. to each, sterilized and inoculated in duplicate with about one gram of soil each as listed below. These cultures were incubated at room temperature for two weeks when they were observed for organisms, and test-

ed for vanillin with Estis' (2) mercuric acid nitrate reagent.

Sub-cultures were prepared similarly except that there was added 2 cc. of normal hydrochloric acid per liter of solution, and that loops of soil culture suspension were used as inoculum for the sub-cultures. These cultures were incubated for three weeks at room temperature, observed for growth of organisms, and tested for vanillin.

TABLE VI.—*Decomposition of Vanillin by Soil Organisms*

	Soil Cultures			Sub-cultures		
Soil	Bacteria	Molds	Vanillin	Bacteria	Molds	Vanillin
1 ------	yes	no	yes	yes	no	no
2 ------	yes	no	yes	yes	no	no
4 ------	yes	no	no	yes	no	no
8 ------	no	yes	yes	no	yes	yes
11 ------	yes	no	no	yes	no	no
13 ------	no	yes	yes	no	no	yes
14 ------	yes	no	no	yes	no	no
15 ------	yes	no	no	yes	no	no
16 ------	yes	no	no	yes	yes	no
17 ------	yes	yes	no	yes	yes	no
18 ------	yes	no	yes	yes	yes	no
20 ------	yes	no	no	yes	no	no
21 ------	no	yes	no	yes	no	no
23 ------	yes	no	no	no	yes	no
24 ------	yes	yes	no	yes	no	no
25 ------	yes	no	no	yes	no	no
26 ------	yes	yes	no	no	yes	no
27 ------	yes	yes	yes	yes	no	yes
28 ------	yes	no	no	yes	no	no
29 ------	yes	no	no	yes	no	no
30 ------	no	yes	yes	no	yes	yes
34 ------	yes	no	no	yes	no	no
37 ------	yes	no	yes	yes	no	no
129 ------	yes	no	no	yes	no	no
Control --	no	no	yes	no	no	yes

From an examination of the data it appears that vanillin was decomposed in 17 of the soil cultures, and in 21 of the sub-cultures. Other similar experiments confirm and extend these results.

Since soils 15, 16, 17, and 18 contain vanillin decomposing organisms it is not difficult to understand why Fraps (3) found injury to growth in only one out of eight crop cultures. Since soils 23, 24, 25 were from the same plots as the soils used by Funchess (4), there is little doubt that the failure of vanillin to depress the yield of oats, except when applied in large amounts immediately after planting, is referable to the activity in his soils of organisms able to decompose the vanillin.

Distribution of Vanillin Decomposing Organisms in Several States

A summary of the accumulated data on the decomposition of vanillin by soil organisms is tabulated below by states from which soils were secured.

TABLE VII.—*Vanillin Decomposing Organisms in Several States*

Source	Soil	Decomposed	Not decomposed	Total
Alabama	1, 2, 3,4,5,6,7,12,22,23,24,25, 27,31,32,33,34,35,36*, 37,38, 40, 41, 42, 43, 129, 130	25	1	26
Virginia	5	1	0	1
Indiana	8*, 9	1	1	2
Michigan	10	1	0	1
Louisiana	11, 12	2	0	2
Texas	13*, 14, 15, 16, 17, 18	5	1	6
Florida	19, 28	2	0	2
Pennsylvania	20, 21, 29, 30*	3	1	4
New York	26	1	0	1
	TOTALS	41	4	45

*Starred numbers represent soils which lack the toxin decomposing organisms.

The summary shows that 41 (91.1%) soils contained vanillin decomposing organisms.

4. CUMARIN

In 1908, Schreiner and Reed (18) reported that cumarin, a lactone of cumaric acid, is extremely poisonous to wheat plants, 100 parts per million causing death in eight days. Later Upson and Powell (28) found that the effect of cumarin on wheat plants in soil is extremely different from its effect in water cultures. Funchess (4) reported that the addition of large amounts of cumarin depresses the yield of oats where oats are planted immediately after the application of the compound. Fraps (3) showed that cumarin when applied 100 parts per million is quite injurious to crops growing on one soil, and somewhat injurious to the crops on three other soils. Robbins (13, 14) found that bacteria destroy the toxicity of cumarin to wheat plants, and that wheat grows better on inoculated soils that contain cumarin than on uninoculated soils.

It has been reported in an earlier section of this bulletin that the optimum reaction for cumarin organisms is 0.4% acid normal to phenolphthalein, and that the nutrient solution described by Robbins (14) or its modification has proven favor-

able for their growth. There remains for consideration the occurrence and distribution of cumarin decomposing organisms.

In order to get some idea of the relative number of soils that contain organisms which decompose cumarin, duplicate flasks containing 50 cc. each of modified Robbins' solution were sterilized and inoculated with soils. After incubation at room temperature for about 3 weeks they were observed for organisms and tested for cumarin by the odor method, which had been found to be reliable for very small traces of cumarin.

Immediately following the testing of the soil cultures another experiment was set up in which sub-cultures similar to the above were prepared by inoculating a pair of flasks of medium, in duplicate, with a fresh suspension of soil number 1, another pair with a suspension of soil number 2 and so on. The results are tabulated below.

TABLE VIII.—*Decomposition of Cumarin by Soil Organisms*

	Soil Cultures			Sub-cultures		
Soil	Bacteria	Molds	Cumarin	Bacteria	Molds	Cumarin
1	no	yes	yes	no	yes	no
2	no	yes	no	no	yes	no
4	no	yes	no	no	yes	no
5	no	yes	no	no	yes	no
9	yes	yes	no	yes	yes	no
11	no	yes	no	no	yes	no
13	yes	yes	no	yes	yes	yes
16	no	yes	no	no	yes	no
17	no	yes	no	no	yes	no
20	no	yes	no	yes	yes	no
23	yes	no	yes	no	no	yes
24	no	yes	no	no	yes	no
25	no	yes	no	no	yes	no
26	no	yes	no	no	yes	no
27	no	yes	yes	no	yes	no
28	no	yes	no	no	no	yes
29	no	yes	no	no	yes	no
31	no	yes	no	no	yes	no
32	no	yes	no	no	yes	no
33	yes	yes	no	yes	yes	no
35	no	yes	yes	no	yes	no
36	no	yes	no	no	yes	no
37	no	yes	yes	no	yes	yes
39	no	yes	no	no	yes	no
40	no	yes	no	no	yes	no
41	no	yes	no	no	yes	no
42	no	yes	no	no	yes	no
43	no	yes	no	no	yes	no
130	no	yes	yes	no	yes	no
Control	no	no	yes	no	no	yes

From these data it appears that decomposition of cumarin occurred in a majority of both soil cultures and sub-cultures. These results show that while few of the soils tested contain bacteria able to decompose cumarin a large majority of them contain molds which decompose this toxin under the conditions imposed.

It may be noted that the dominance of mold decomposition is probably due to the rather sour condition of the medium, and also that in other experiments where there was less acidity bacteria were effective in decomposing cumarin.

There is little doubt that the different effects of cumarin on wheat plants in soil and solution cultures reported by Upson and Powell (28) are to be referred to the functioning of the cumarin decomposing organisms in one case and not in the other, depending on conditions such as the presence or absence of suitable salts, and favorable reaction. It is not surprising that Funchess (4) found no injury and even benefit to corn and oats except when the crop was planted immediately after the application of large quantities of cumarin, as soils 24 and 25 are from the plots from which he obtained soils for his work, and contain organisms which soon change the toxin to an inert or, perhaps, useful substance for the plant.

Since cumarin decomposing organisms were found in each of Fraps' (3) soils in which cumarin was more or less injurious, we can only interpret his results on the assumption that the reaction or fertilizer contents of these soils were not favorable for the decomposition of the toxin.

Distribution of Cumarin Decomposing Organisms in Several States

The accumulated data on the decomposition of cumarin by soil organisms of several states are summarized in the following table:

TABLE IX.—*Cumarin Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2, 3, 4,6,7,22,23*,24,25,27, 31, 32, 33,34,35,36,37,38,39, 40, 41, 42, 43, 129, 130	25	1	26
Virginia	5	1	0	1
Indiana	8*, 9	1	1	2
Michigan	10	1	0	1
Louisiana	11, 12	2	0	2
Texas	13, 14, 15, 16, 17, 18	6	0	6
Florida	19, 28	2	0	2
Pennsylvania	20, 21, 29, 30	4	0	4
New York	26	1	0	1
	TOTALS	43	2	45

*Cumarin decomposing organisms lacking.

Totaling these figures it appears that 43 (95.5%) of the 45 soils examined contained decomposing organisms, while two (4.4%) of the soils examined did not contain them.

5. CINNAMIC ACID

Though cinnamic acid has not been isolated from soils, it is found in the bark of plants. Its esters occur in the leaves of enough plants to warrant an investigation of its effect on plants and a search for means of destroying it. It is found in the bark of *Toluifera Balsamum, Styrax Benzoin,* and *Cinnamomium zeylanicum.* The esters of cinnamic acid occur in the leaves of *Erythroxylon Coca and Thea sinensis.*

Schreiner and Reed (18) found cinnamic acid strongly toxic. and sodium cinnamate moderately toxic to growth of wheat seedlings, especially their roots. True (26) found that cinnamic acid killed the root tips of lupine in twenty-four hours when 12 parts per million were used. He also found that of the sodium salts of organic acids sodium cinnamate was the most toxic to lupine seedlings. Injury occurred when 184 parts per million were used.

Since cinnamic acid has been reported toxic to both wheat and lupine seedlings it seems that the next logical step is to determine the probability of eliminating this substance from the soil. There are the following possibilities: root excretions may render the cinnamic acid harmless; fertilizer applications may neutralize the acid and cause precipitation; or micro-organisms under favorable conditions may destroy it as they use the cinnamic acid as the source of carbon, and the fertilizer may merely complete the favorable conditions.

In order to test the ability of soil organisms to decompose cinnamic acid Robbins' solution to which 0.5 grams of cinnamic acid has been added, was prepared, distributed into small flasks, sterilized, and inoculated with soils from different sources. After incubating at room temperature for 15 days, observations on the character of growth were made and the medium in each flask tested with potassium permanganate for cinnamic acid.

TABLE X.—*Decomposition of Cinnamic Acid by Soil Organisms*

Soil	Bacteria	Molds	Cinnamic acid
1	yes	no	yes
2	yes	yes	yes
3	yes	yes	no
4	yes	no	no
5	yes	no	no
6	yes	no	no
7	yes	no	no
8	no	no	yes
9	yes	yes	no
10	yes	yes	no
11	yes	yes	no
12	yes	yes	no
13	yes	no	no
14	yes	yes	no
15	yes	yes	no
16	yes	no	yes
17	yes	yes	no
18	yes	no	no
19	yes	yes	no
20	yes	yes	no
21	yes	no	no
22	yes	no	no
23	yes	yes	no
24	yes	yes	no
25	yes	yes	no
26	yes	yes	no
27	yes	yes	no
28	no	yes	no

From the above results it is evident that a large percentage of the soils tested contain organisms which decompose cinnamic acid. These results have been extended by subsequent tests.

The Distribution of Cinnamic Acid Decomposing Organisms in Several States

A summary of the accumulated data on decomposition of cinnamic acid by soil organisms of several states follows in table XI:

TABLE XI.—*Cinnamic Acid Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2*, 3,4,6,7,22,23,24,25,27, 31,32,33,34*,35,36*,37*,38*, 39,40*,41*,42*,43,129*,130*	15	11	26
Virginia	5	1	0	1
Indiana	8*, 9	1	1	2
Michigan	10	1	0	1
Louisiana	11, 12	2	0	2
Texas	13*, 14, 15, 16*, 17, 18	4	2	6
Florida	19, 28	2	0	2
Pennsylvania	20, 21, 29, 30	4	0	4
	TOTALS	31	14	45

*Cinnamic acid decomposing organisms lacking.

The summary shows that 31, or 69%, of the forty-five soils examined, contained cinnamic acid decomposing organisms.

6. QUINONE

There is nothing in print, known to the writer, to explain the great difference in the toxicity of soil cultures and solution cultures containing quinone. Most of Fraps' (3) soil cultures containing quinone in liberal amounts show little or no injury to the plants grown in them, while the quinone solution cultures of Schreiner, Reed and Skinner (20) show distinct injury to crop plants, even when small amounts of quinone are present. In a recent article Massey (10a) points out that the injurious effects of a related substance, juglone, are limited to the region close to the roots of the walnut.

On account of the reported difference in its toxicity, its wide distribution in plants, such as hickories and pecans, and the probability that quinone frequently occurs in soils, more information concerning its fate in soils and in the solution cultures is very desirable. Even though the injurious effect of quinone on plants may be diminished by absorption, the quinone decomposed by chemical processes or removed by leaching, it is more probable that its harmful effects are prevented by biological agencies.

The results obtained by Robbins (14) with vanillin, cumarin, and pyridine give ground for the supposition that quinone may be decomposed by bacteria or molds. An attempt to support this supposition is reported below.

To determine whether the microorganisms of the soil can reduce or decompose quinone several Erlenmeyer flasks containing 50 cc. of a modification of Robbins' solution, having a reaction of 0.8% acid normal, with 0.5 gram of quinone per liter as

the source of carbon, were each inoculated in duplicate with one gram of soil respectively, from various sources. These cultures were allowed to incubate at room temperature for two weeks, when they were tested for the presence of quinone.

Sub-cultures prepared as for soil cultures, except that inoculations were made from the soil cultures, were incubated at room temperature for 21 days, observed for growth of organisms, and tested by the odor test, with and without ferric chloride, for the presence of quinone. The results are shown in the table which follows:

TABLE XII.—*Decomposition of Quinone by Microorganisms*

	Soil Cultures—Reaction 0.8%				Sub-cultures—Reaction 0.6%			
	Growth		Quinone		Growth		Quinone	
Soil	Bacteria	Molds	Odor	$FeCl_3$	Bacteria	Molds	Odor	$FeCl_3$
1 --	no	yes	no	yes	no	yes	no	no
3 --	yes	yes	no	no	no	yes	yes	yes
8 --	no	yes	no	yes	no	yes	no	yes
9 --	yes	yes	no	yes	no	yes	no	no
13 --	no	yes	no	yes	no	yes	no	
14 --	yes	yes	no	yes	no	yes	no	
16 --	no	yes	no	yes	no	yes	no	
17 --	yes	yes	no	yes	no	yes	no	
19 --	yes	yes	yes	yes	no	yes	no	
21 --	yes	yes	no	yes	no	yes	no	
22 --	no	yes	no	yes	no	yes	no	
23 --	no	yes	no	yes	no	yes	no	
24 --	no	yes	no	yes	no	yes	no	
25 --	no	yes	no	yes	no	yes	no	
26 --	no	yes	no	yes	no	yes	no	
27 --	yes	yes	no	yes	no	yes	no	
28 --	no	yes	no	yes	no	yes	no	
31 --	yes	yes	no	yes	no	yes	no	
32 --	no	yes	no	no	no	yes	no	
33 --	no	yes	no	yes	no	yes	no	
34 --	no	yes	yes	yes	no	yes	yes	
35 --	no	yes	no	yes	no	yes	no	
36 --	yes	yes	no	yes	no	yes	no	
38 --	no	yes	no	yes	no	yes	no	
39 --	no	yes	no	no	no	yes	yes	
40 --	no	yes	no	yes	no	yes	no	
41 --	yes	yes	no	yes	no	yes	no	
42 --	yes	yes	no	yes	no	yes	no	
43 --	no	yes	no	yes	no	yes	no	
129 --	yes	yes	no	yes	no	yes	no	
130 --	no	yes	no	yes	no	yes	no	
Blank	no	no	yes	yes	no	no	yes	

Destroyed by an accident

It appears from these results that certain organisms are able to decompose quinone in solution cultures. It is to be noted that bacteria grew in some and molds in many cultures.

It is apparent that the bacteria in the soil cultures obtained carbon from the soil used for inoculation instead of from the quinone. It will be noticed that a good growth of mold was secured in sub-cultures but none of bacteria. Further, it will be observed that in some instances, e. g. soils 1 and 9, the quinone has been decomposed rather than changed to hydroquinone since the solution has no odor of quinone either before or after treating with ferric chloride. The ferric chloride readily oxidizes the odorless hydroquinone to the pungent smelling quinone. In other cases, e. g. soil 8, the quinone has been merely reduced to hydroquinone as is indicated by the test with ferric chloride.

Particular attention should be called to soils 15, 16, 17 and 18, which were used by Fraps (3), who found that corn grown on cultures of soil 16 containing respectively 100 and 200 parts per million of quinone was not injured. Where 300 parts per million were used the yield was reduced 39%. In case of soil 17 three hundred parts per million reduced the yield of corn 27%. In case of soil 15 one hundred parts per million reduced the yield of sorghum 95.8% while one hundred parts per million of quinone applied weekly beginning ten days after planting on soil 18 reduced the yield of sorghum 18%.

It appears that molds in soils 16 and 17 reduced quinone, while the organisms present in soils 15 and 18 did not reduce it. Apparently there were no organisms present in soil 15 able to decompose or reduce the quinone in Fraps' soil cultures. Soil 16 probably contained organisms which reduced quinone before injury occurred to his corn plants.

In soils 17 and 18 the relation is not quite clear. Probably the conditions were not favorable for vigorous growth and activity of the quinone decomposing organisms in soil 17 when Fraps worked with it, though later they were able to decompose quinone in our solution cultures. Probably the organisms were present in limited numbers and under unfavorable conditions when Fraps found an injury of 18% to sorghum plants grown in soil 18, and died before they reached this laboratory. The injury to crop plants of Schreiner, Reed and Skinner (20) may be referred to the lack of quinone decomposing organisms.

From the soil cultures and the sub-cultures it appears that many soils contain organisms able to reduce quinone to the less harmful hydroquinone, and that some soils contain organisms which decompose quinone into a still different substance that is not easily reoxidized to quinone.

Distribution of Quinone Decomposing Organisms in Several States

The accumulated data on the decomposition of quinone by soil organisms found in several states give the results shown in Table XIII which follows:

TABLE XIII.—*Quinone Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2, 3, 4*, 6*, 7*, 22, 23, 24, 25, 27, 31, 32, 33, 34*, 35, 36, 37*, 38, 39, 40, 41, 42, 43, 129, 130*	20	6	26
Virginia	5*	0	1	1
Indiana	8, 9	2	0	2
Michigan	10*	0	1	1
Louisiana	11*, 12*	0	2	2
Texas	13, 14, 15*, 16, 17, 18*	4	2	6
Florida	19, 28	2	0	2
Pennsylvania	20*, 21, 29*, 30*	1	3	4
New York	26	1	0	1
	TOTALS	30	15	45

*Quinone decomposing organisms lacking.

The summary shows that 30, or 66.6%, of the soils, examined contained quinone decomposing organisms.

7. HYDROQUINONE

While hydroquinone has not been definitely isolated from soils, it is likely to be found wherever quinone occurs, and conditions for reduction are favorable. According to Smith (25) hydroquinone is readily obtained by the reduction of p-quinone. Czapek (1a) says it is found by the hydrolysis of arbutin which occurs in many Ericaceae, such as Kalima. It has been found by Riviere and Bailhache (12) in the leaf buds, and by Lippmann (9) in the fresh pith of the pear. Since this substance occurs in common plants it may reach the soil through their falling leaves and twigs.

True and Hunkel (27) found that for lupine roots hydroquinone was more toxic than its two isomers, pyrocatechin and resorcinol. Schreiner and Reed (19) report that hydroquinone and pyrocatechin have about equal toxicity, less than quinone and greater than resorcinol for wheat plants. On account of its relation to quinone and its reported toxicity to wheat and lupine seedlings it has been used as a source of carbon for microorganisms in soils, and its susceptibility to decomposition has been determined.

In order to determine whether organisms in soils can use hydroquinone as the source of carbon, and to determine whether any of them are able to decompose this toxin, modified Robbins' solution containing 0.5 gram hydroquinone per liter and having a reaction of 0.4% acid normal to phenolphthalein was distributed, 50 cc. to the flask and sterilized. These flasks were inoculated in pairs with the soils below and placed to incubate in darkness at room temperature for about three weeks.

The tests for hydoquinone consist of heating 10 to 12 cc. of the clear solution in an evaporating dish and smelling. Absence of the pungent odor was taken to mean that the hydroquinone was not oxidized to quinone. If the pungent odor of quinone was obtained after addition of $FeCl_3$ and heating it was assumed that the hydroquinone persisted.

TABLE XIV.—*Decomposition of Hydroquinone by Soil Organisms*

Soil	Organisms		Quinone	
	Bacteria	Molds	Odor	Ferric Chloride
12	no	no	no	yes
21	no	yes	no	yes
22	no	yes	no	yes
23	no	yes	no	yes
25	no	yes	no	yes
26	no	yes	yes	yes
27	no	yes	no	yes
28	no	yes	no	yes
33	no	yes	yes	yes
34	no	yes	yes	yes
35	no	yes	no	no
36	no	yes	no	yes
42	no	yes	no	yes
Control	no	no	yes	yes

From these results it appears that several soils contain molds which can use hydroquinone as the source of carbon, even though none of the soils contain bacteria which are able to utilize this substance. The results indicate that soils 26, 33, and 34 contain organisms able to oxidize this toxin to quinone, while soil 35 contains organisms which decompose it so completely that it does not give the test for quinone either before or after treating with ferric chloride. Other experiments supplement and extend this one, and show about one third of the soils tested contain organisms which decompose hydroquinone.

Hydroquinone Decomposing Organisms in Several States

Summarized data for the several states are presented in Table XV below.

TABLE XV.—*Hydroquinone Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2*, 3,4*,6*,7*,22,23,24,25, 27*, 31*,32*,33*,34*,35,36*, 37*, 38,39*,40,41*,42*,129*, 130*	9	17	26
Virginia	5*	0	1	1
Indiana	8, 9	2	0	2
Michigan	10	1	0	1
Louisiana	11*, 12*	0	2	2
Texas	13*, 14, 15, 16, 17*, 18*	3	3	6
Florida	19, 28*	1	1	2
Pennsylvania	20*, 21, 29*, 30*	1	3	4
New York	26*	0	1	1
	TOTALS	17	28	45

*Hydroquinone decomposing organisms lacking.

These results show that 17, or 37.7%, of the soils contained hydroquinone decomposing organisms.

8. RESORCINOL

Whether free resorcinol finds its way into soils on which crop plants grow or whether combined resorcinol occurs in soils has not been definitely reported in available literature. It has not been isolated though there are some indications that it occurs in soils.

Regardless of its occurrence in soils, True and Hunkel (27) found that the growth of lupine roots was prevented by a solution containing 500 parts per million, and Schreiner and Reed (19) found that the lowest concentration causing death was 1000 parts per million.

Since resorcinol has been found to be toxic to more than one kind of plant it seemed desirable to determine the fate of this toxin under the action of microorganisms by means of the following experiments.

Small flasks of Robbins' solution to which 0.5 gram per liter of resorcinol had been added, were prepared in the usual way, inoculated respectively with soils from different sources, and incubated at room temperature for two weeks. The results are tabulated under "Soil Cultures." For the sub-cultures a medium like the above was prepared and 0.2 gram of monobasic calcium phosphate per liter added. This was distributed in

small flasks, inoculated in the usual manner, and placed to incubate at room temperature for about two weeks. The results are tabulated under "Sub-cultures."

TABLE XVI.—*Decomposition of Resorcinol by Soil Organisms*

	Soil Cultures No Calcium			Sub-cultures Monobasic Calcium Phosphate		
Soil	Bacteria	Molds	Resorcinol	Bacteria	Molds	Resorcinol
1	yes	no	no	no	yes	no
2	yes	yes	no	no	yes	no
3	no	yes	yes	no	yes	no
4	yes	yes	no	no	yes	no
5	no	yes	no	no	yes	no
6	yes	yes	no	no	yes	no
7	yes	yes	yes	no	yes	yes
8	no	yes	yes	no	yes	no
9	no	yes	no	no	yes	no
10	no	yes	no	no	yes	no
11	yes	yes	no	no	yes	no
12	yes	no	no	no	yes	no
13	yes	no	no	no	yes	no
14	yes	no	no	no	yes	no
15	yes	no	no	no	no	yes
16	yes	yes	no	no	no	yes
17	yes	yes	no	no	yes	no
18	yes	yes	no	no	yes	no
19	yes	yes	no	no	yes	no
20	no	yes	no	no	yes	no
21	no	yes	no	no	yes	no
22	yes	yes	no	no	yes	no
23	no	yes	no	no	yes	no
24	no	yes	no	no	yes	no
25	no	yes	no	no	yes	no
26	yes	yes	no	no	yes	no
27	no	yes	no	no	yes	no
Control	no	no	yes	no	no	yes

Other similar experiments show that most of the soils tested contain organisms (mostly molds) able to decompose resorcinol even in the presence of the monobasic calcium phosphate.

Distribution of Resorcinol Decomposing Organisms in Several States

The accumulated data on the decomposition of resorcinol by soil organisms in several states are given in Table XVII which follows:

TABLE XVII.—*Resorcinol Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1,2,3,4,6,7,22,23,24,25,27,31, 32, 34*, 35, 36, 37,38*,39,40, 41, 42, 43, 129, 130	23	2	25
Virginia	5	1	0	1
Indiana	8, 9	2	0	2
Michigan	10	1	0	1
Louisiana	11, 12	2	0	2
Texas	13, 14, 15, 16, 17, 18	6	0	6
Florida	19, 28	2	0	2
Pennsylvania	20, 21, 29, 30	4	0	4
New York	26	1	0	1
	TOTALS	42	2	44

*Resorcinol decomposing organisms absent.

Summarizing these data it appears that of the 44 soils examined 42 (95.4%) contained resorcinol decomposing organisms.

III. THE DECOMPOSTION OF NITROGENOUS TOXINS BY SOIL ORGANISMS

1. CAFFEIN

In the case of nitrogenous toxins it seems that a somewhat different substratum might be required than that best suited to organisms which decompose non-nitrogenous toxins. At least other nitrogenous compounds must be omitted, and perhaps some provision made for a supply of organic carbon. The nitrogenous compound chosen to illustrate the general conditions for the decomposition of nitrogenous toxins is caffein. It has been chosen largely because it has been reported by Lutz (10) as toxic to higher plants.

According to Czapek (1a) this purine base is found in the leaves of tea, in the seeds of coffee, and in parts of various other plants. Through the decomposition of these parts of plants it may readily reach the soil about dwellings.

Preliminary experiments show that calcium sulphate and low acidity are beneficial, while monobasic calcium phosphate is detrimental to the growth of caffein organisms.

To settle the question of available carbon three sets of cultures were prepared. The stock medium for each set consisted of:

Dipotassium phosphate	0.5 gram
Potassium chloride	0.2 gram
Magnesium sulphate	0.2 gram
Calcium sulphate	0.2 gram
Ferric chloride	Trace
Distilled water	1000 cc.
Caffein	0.5 gram
Reaction	0.1%

This medium has the same composition as that tested for non-nitrogenous toxins except that $NaNO_3$ and NH_4Cl are omitted while dextrose and the toxin are added.

It will be observed that there are two organic compounds in this medium—dextrose and caffein. This situation offers several possibilities. The caffein may furnish both nitrogen and carbon, or only the necessary nitrogen. The addition of dextrose may or may not be necessary for the life of the organisms. Again, the dextrose may be preferred and consumed by the soil organisms before the caffein is attacked and thus retard the decomposition of the caffein.

For the first set of cultures this stock solution was distributed to flasks, sterilized and inoculated in the usual manner. For the second set 0.5 gram dextrose was added to the stock solution and treated as above. To the stock solution for the third set 5 grams of dextrose were added and treated as above. After about 2 weeks the results as tabulated in Table XVIII were obtained.

TABLE XVIII.—*Carbon for Caffein Decomposing Organisms*

	No Dextrose		.5 Gram Dextrose		5 Grams Dextrose	
Soil	Bacteria	Molds	Bacteria	Molds	Bacteria	Molds
7	no	yes	yes	no	yes	yes
9	no	yes	yes	yes	no	yes
20	no	yes	yes	yes	yes	yes
21	yes	no	yes	yes	yes	yes
22	yes	yes	yes	yes	yes	yes
25	no	yes	yes	yes	yes	yes
26	no	yes	no	yes	no	yes
37	no	yes	no	yes	no	yes
42	yes	no	yes	no	no	yes
43	yes	no	no	no	no	yes
Control	no	no	no	no	no	no

From these results it appears that caffein bacteria grow better where dextrose is present than where it is absent, and that a less amount than 5 grams per liter of medium yields growth of bacteria in the greatest number of cultures. It appears that caffein molds thrive better in the presence of the larger amount of dextrose.

Decomposition of Caffein by Soil Organisms

Having found that the growth of caffein organisms is hindered by the presence of monobasic calcium phosphate and promoted by the presence of dextrose, the following medium was prepared in the usual manner and inoculated with several soils and incubated for ten days at room temperature:

Dipotassium phosphate	0.5 gram
Potassium chloride	0.2 gram
Magnesium sulphate	0.2 gram
Calcium sulphate	0.2 gram
Ferric Chloride	Trace
Dextrose	2.0 grams
Distilled water	1000 cc.
Reaction	0.2%

The growth of the organisms and the decomposition of caffein as shown by the murexide test is recorded in Table XIX under the heading "Soil Cultures." Sub-cultures were made in a medium of the same composition and with inoculum consisting of a loop of suspension from each soil culture. These were incubated at room temperature for about 4 weeks, when they were observed for growth, and examined for caffein and the decomposition of the caffein as recorded in Table XIX under "Sub-cultures."

TABLE XIX.—*Decomposition of Caffein by Soil Cultures*

	Soil Cultures			Sub-cultures		
Soil	Bacteria	Molds	Caffein	Bacteria	Molds	Caffein
33	no	yes	no	no	yes	no
34	no	yes	yes	no	yes	yes
37	yes	yes	no	yes	yes	no
38	yes	yes	no	yes	yes	no
39	no	yes	no	no	yes	no
40	yes	yes	no	yes	yes	no
41	yes	yes	no	yes	yes	no
42	yes	yes	no	yes	yes	no
43	no	yes	no	no	yes	no
129	yes	yes	no	yes	yes	no
130	no	yes	no	no	yes	no
Control	no	no	yes	no	no	yes

It is evident that some bacteria and many molds are able to grow with caffein as the source of nitrogen, and that a majority of the soils in this test contain organisms able to decompose this substance.

The reaction best suited for the growth of caffein decomposing organisms was not determined quite as precisely as was desirable. The tests made indicate a rather wide tolerance with the optimum at about .1% acid normal.

The Distribution of Caffein Decomposing Organisms in Several States

The distribution of the organisms which decompose caffein presents a somewhat different case from that of vanillin and resorcinol. Neither in Alabama nor in other states are there as many soil organisms which decompose caffein. The data for the soils of several states are given in Table XX which follows:

TABLE XX.—*Caffein Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1*,2*,3*,4,6,7,22,23,24*,25*, 27, 31, 32, 33*,34*,35,36,37, 38,39,40,41,42,43,129,130	19	7	26
Virginia	5	1	0	1
Indiana	8*, 9*	0	2	2
Michigan	10*	0	1	1
Louisiana	11, 12*	1	1	2
Texas	13, 14, 15*, 16*, 17, 18	4	2	6
Florida	19*, 28	1	1	2
Pennsylvania	20, 21, 29*, 30	3	1	4
New York	26*	0	1	1
	TOTALS	29	16	45

*Caffein decomposing organisms absent.

From these figures it appears that 29, or 64.4%, of the 45 soils contained caffein decomposing organisms.

2. PYRIDINE

The alkaloid pyridine has been included in these studies mainly because is has already received consideration as a factor in soil fertility. In 1899 its hydrochloride was found by Lutz (10) to be injurious to seedlings of maize and curcurbits. In 1906 Shorey (22) reported the isolation of pyridine from soil by distillation and ether extraction. The next year Schreiner, Reed, and Skinner (20) found pyridine injurious to wheat seedlings in solution cultures. In 1916 Funchess (4) found pyridine highly beneficial to both oats and corn in all soils tested. The next year he (5) reported that pyridine was decomposed in limed

soils, while Robbins (14) found that it was decomposed by pure cultures of soil organisms in solution cultures. There remains a study of the conditions and products of its decomposition, as well as the general occurrence and distribution of pyridine decomposing organisms.

The Occurrence of Pyridine Decomposing Organisms

Even though Robbins (14) demonstrated the decomposition of pyridine by soil organisms he reports no attempt to determine how commonly it occurs, nor does he give any idea of the distribution of the organisms.

A preliminary test showed that few of the soils contained bacteria able to use pyridine as the main source of carbon, while most of them contained molds able to grow with pyridine as the source of carbon.

In the attempt to determine the prevalence of soil organisms which cause the disappearance of pyridine in nutrient solutions, the stock solution used for cultures of caffein decomposing organisms was distributed to Erlenmeyer flasks, 50 cc. to the flask. One drop of pyridine was added to each flask just before it was sterilized. After the inoculation of the 47 pairs of flasks, with one gram of soil to a pair, the cultures were allowed to incubate at room temperature for about three weeks. Then they were examined for growth of bacteria and molds and tested for the odor of pyridine. This test was found to be more sensitive than any other, being effective when pyridine was present in a dilution of 1 to 50,000. Since it existed only infrequently in incubated cultures fatigue of the sense of smell did not occur. Decomposition of pyridine occurred in both soil cultures and sub-cultures 24, 25, 26, 27, 29, 32, 36, 39, 42, 43, 45, 46, and 129, but not in soil cultures and sub-cultures 30, 33, 35, 38, 40, 41, and 44. All together, according to Table XXI, and the above statement, growth of bacteria occurred in 46, growth of molds in 20, and decomposition of pyridine occurred in 41 soil cultures.

There was growth of bacteria in a total of 11, growth of molds in 47, and decomposition of pyridine in 38 sub-cultures. We may refer the difference of growth of bacteria and molds to the reactions of the nutrient solutions and conclude that most of the soils under investigation contain bacteria and molds able to decompose pyridine when the reaction is favorable. This conclusion is supported by Table XXI which follows.

TABLE XXI.—*Decomposition of Pyridine by Soil Cultures*

Soil	Soil Cultures			Sub-cultures		
No.	Bacteria	Molds	Toxin	Bacteria	Molds	Toxin
1	yes	yes	no	no	yes	yes
2	yes	yes	yes	yes	yes	yes
3	yes	yes	no	no	yes	no
4	yes	yes	no	yes	yes	no
5	yes	yes	yes	no	yes	yes
6	yes	yes	no	no	yes	no
7	yes	yes	no	no	yes	yes
8	no	yes	no	no	yes	no
9	yes	yes	no	no	yes	no
10	yes	yes	no	no	yes	no
11	yes	yes	no	no	yes	no
12	yes	yes	no	no	yes	no
13	yes	yes	no	no	yes	no
14	yes	no	no	no	yes	no
15	yes	yes	no	no	yes	no
16	yes	yes	no	no	yes	no
17	yes	no	no	yes	yes	no
18	yes	yes	yes	no	yes	yes
19	yes	no	yes	no	yes	yes
20	yes	no	no	no	yes	no
21	yes	no	no	no	yes	no
22	yes	yes	no	yes	yes	no
23	yes	yes	no	no	yes	no
28	yes	yes	no	no	yes	yes
34	yes	yes	yes	no	yes	no
37	yes	yes	no	no	yes	yes
46	yes	yes	no	yes	yes	no
Check	no	no	yes	no	no	yes

Nitrates in the Experimental Soils

In order that it might be known definitely whether nitrates were already present in these soils, or whether the soil organisms nitrified pyridine in the culture, the soils were tested qualitatively for nitrates by the Molisch (11) method. A hot water filtrate was tested in the same manner. The results are shown in Table XXII.

TABLE XXII.—*Nitrates in Test Soils*

Soil Solution				A hot water filtrate of each soil			
Soil	Nitrate	Soil	Nitrate	Soil	Nitrate	Soil	Nitrate
1	no	22	no	1	yes	22	no
2	no	23	no	2	no	23	no
3	no	24	no	3	no	24	yes
4	yes	25	no	4	yes	25	yes
5	no	26	no	5	no	26	no
6	yes	27	no	6	yes	27	no
7	yes	29	no	7	yes	29	no
8	no	30	yes	8	no	30	no
9	yes	31	yes	9	yes	31	yes
10	yes	32	no	10	no	32	no
11	no	34	no	11	yes	34	no
12	no	36	no	12	yes	36	no
13	no	39	no	13	no	42	yes
14	no	42	yes	14	no	43	no
15	no	43	no	15	no	46	no
16	yes	46	no	16	yes	129	yes
17	yes	129	no	17	yes	130	no

An examination of the above table brings out the fact that nitrates were detected by both methods in ten soils.

The Decomposition and Nitrification of Pyridine

Since nitrification of this substance in soils had been suggested by earlier investigators it was thought desirable to test for nitrates in the nutrient solution in which decomposition of pyridine occurred. Cultures were prepared and treated as indicated in the preceding experiment, except that a portion of the substratum was tested for the presence of nitrates by means of the diphenylamine-sulphuric acid reagent. One drop of this solution was allowed to run down the side of the dish containing the solution to be tested, when the appearance of a blue color indicated the presence of nitrates. Table XXIII is a record of this work.

TABLE XXIII.—*Decomposition and Nitrification of Pyridine*

	Soil Cultures				Sub-cultures			
Reaction:	The solution was 0.43% acid normal with methyl orange indicator before the toxin was added.				This solution was 0.2% acid normal before the toxin was added.			
No.	Bacteria	Molds	Toxin	NO_3	Bacteria	Molds	Toxin	NO_3
1	yes	yes	no	no	yes	yes	yes	no
2	no	yes	yes	no	no	yes	yes	no
3	yes	yes	no	no	yes	yes	no	no
4	yes	yes	no	no	yes	yes	no	no
5	yes	yes	yes	no	yes	yes	no	no
6	yes	yes	no	no	yes	yes	no	no
7	yes	yes	no	no	yes	yes	no	no
8	yes	yes	no	no	yes	yes	no	no
9	yes	yes	no	no	yes	yes	no	no
10	yes	yes	yes	no	yes	yes	no	no
11	yes	yes	no	no	yes	yes	no	no
12	yes	yes	no	no	yes	yes	no	no
13	no	yes	yes	no	no	yes	yes	no
14	yes	yes	no	yes	yes	yes	no	no
15	yes	yes	no	no	no	yes	yes	no
16	yes	yes	no	no	yes	yes	no	no
17	yes	yes	no	no	yes	yes	no	no
20	yes	yes	no	no	yes	yes	no	no
21	yes	yes	no	yes	yes	yes	no	no
22	yes	yes	no	no	yes	yes	no	no
23	yes	yes	no	no	yes	yes	no	no
24	yes	yes	no	no	yes	yes	no	no
25	yes	yes	no	yes	yes	yes	yes	no
26	yes	yes	no	no	yes	yes	no	no
27	yes	yes	no	no	yes	yes	no	no
29	yes	yes	no	no	yes	yes	no	no
30	yes	yes	no	yes	yes	yes	yes	no
31	yes	yes	no	no	yes	yes	no	no
32	yes	yes	no	no	yes	yes	no	no
34	yes	yes	yes	yes	yes	yes	yes	no
36	yes	yes	no	yes	yes	yes	no	no
37	yes	yes	no	no	yes	yes	no	no
39	yes	yes	no	yes	yes	yes	no	no
42	yes	yes	no	no	yes	yes	no	no
43	yes	yes	no	no	yes	yes	no	no
46	yes	yes	no	no	yes	yes	no	no
129	yes	yes	no	yes	yes	yes	no	no
130	yes	yes	no	yes	yes	yes	no	no
Check	no	no	yes	no	no	no	yes	no

The results recorded above show the presence of nitrates in but few soil cultures, viz., 14, 21, 25, 30, 34, 36, 39, 129, 130, and in no sub-cultures. A comparison of these results with those

where soils were tested with nitrates shows that nitrification of pyridine occurred only in cultures 14, 36, and 39. Incidentally, these results confirm those of the preceding experiment as to the decomposition of pyridine.

Thus it appears that a high percentage of soils contained organisms able to decompose pyridine when available carbon is present and the reaction is not far removed from neutral; and that a small percentage of the soils contained organisms which nitrified the pyridine under the conditions imposed.

The collected data for the soils of the several states are given in Table XXIV.

TABLE XXIV.—*Pyridine Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2*, 3, 4,6,7,22,23,24,25,27, 31,32,33*,34*,35*,36,37,38*, 39, 40*, 41*, 42, 43, 44*, 129 130	19	8	27
Virginia	5*	0	1	1
Indiana	8, 9	2	0	2
Michigan	10	1	0	1
Louisiana	11, 12, 45, 46, 48	5	0	5
Texas	13, 14, 15, 16, 17, 18*	5	1	6
Florida	19*, 28	1	1	2
Pennsylvania	20, 21, 29, 30*	3	1	4
New York	26	1	0	1
	TOTALS	37	12	49

*Pyridine decomposing organisms lacking.

The summary shows that of the 41 soils from the various parts of the United States, 37, or 75.5%, contained pyridine decomposing organisms.

3. PIPERIDINE

Piperidine is a nitrogenous base, chemically known as pentamethylene imide or hexahydropyridine. It occurs in combination in the pepper plant and forms the nucleus for many alkaloids. Like pyridine it is included in these investigations because it has already received some consideration as a factor in soil fertility.

In 1907 Schreiner, Reed and Skinner (20) reported it as harmful to wheat seedlings in solution cultures containing 25 parts per million, though one part per million was beneficial. Funchess (5) reported that the "nitrification of piperidine was greatly increased by lime."

The investigation of the behavior of piperidine in solution cultures was carried on similarly to that for pyridine. The same stock solution, indicators, incubation period, soils, etc., were employed for its study.

A preliminary experiment indicated the need of a readily available carbohydrates as the source of carbon for the decomposing organisms.

The Decomposition of Piperidine by the Microorganisms

The modification of Robbins' solution used for pyridine studies was employed. The reaction of the nutrient solution for the soil cultures was 0.1% acid normal before the toxin was added. The reaction of the solution for the sub-cultures was 0.8% acid normal before the toxin was added. This solution was distributed to flasks, sterilized, and inoculated in the usual manner. After incubating for three weeks at room temperature the cultures were observed for growth of organisms and tested for the presence of piperidine as reported in Table XXV.

TABLE XXV.—*Decomposition of Piperidine by Soil Organisms*

	Soil Cultures			Sub-cultures		
No.	Bacteria	Molds	Toxin	Bacteria	Molds	Toxin
1	yes	yes	yes	yes	yes	no
2	yes	yes	yes	no	yes	no
3	yes	yes	yes	no	yes	yes
4	yes	yes	yes	no	yes	no
5	yes	yes	no	no	yes	no
6	yes	yes	yes	no	yes	no
7	yes	yes	no	yes	yes	no
8	yes	yes	no	no	yes	no
9	yes	yes	no	no	yes	no
10	yes	yes	no	no	yes	no
11	yes	yes	no	yes	yes	no
12	yes	yes	no	no	yes	no
13	yes	yes	no	no	yes	no
14	yes	yes	no	yes	yes	no
15	yes	yes	no	yes	yes	no
16	yes	yes	no	yes	yes	no
19	yes	yes	no	no	yes	no
20	yes	yes	no	yes	yes	no
21	yes	yes	yes	no	yes	no
22	yes	yes	no	no	yes	no
23	yes	yes	no	no	yes	no
24	yes	yes	yes	yes	yes	yes
26	yes	yes	yes	no	yes	no
28	yes	yes	yes	yes	yes	yes
44	yes	yes	yes	no	yes	no
Check	no	no	yes	no	no	yes

Besides those given in Table XXV decomposition of piperidine occurred in both soil and sub-cultures 25, 27, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 42, 43, 45, 46, and 129, but not in soil and sub-cultures 28, 32, 40, and 41. Growth of bacteria occurred in 43, growth of molds in all, and decomposition of piperindine in 21 of the 45 soil cultures. Growth of bacteria occurred in 22, growth of molds in all, and decomposition of piperidine in 39 of the 45 sub-cultures. From these data it appears that most soils contain organisms which decompose piperidine under favorable conditions.

Nitrification of Piperidine by Soil Organisms in Solution Cultures

An attempt was made to demonstrate the change of piperidine to nitrates both in soil cultures and in sub-cultures under favorable conditions as to reaction. The experiment was set up and carried out in the usual manner in every detail. An examination of the data showed that only the duplicate cultures of soil 129 gave a test of nitrates after the usual treatment. Since it has been shown already that soil 129 contains detectable quantities of free nitrates it is not proper to consider this a case of nitrification. Instead we are forced to admit that the organisms of 38 soils did not change the piperidine to nitrates under the conditions of the experiment which included both soil cultures and sub-cultures.

Distribution of Piperidine Decomposing Organisms in Several States

The accumulated data on the decomposition of piperidine by organisms occurring in the soils of several states are recorded in Table XXVI which follows.

TABLE XXVI.—*Piperidine Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2,3*,4,6,7,22,23,24*,25,27, 31, 32*,33,34,35,36,37,38,39, 40*,41*,42,43,44*,129,130	21	6	27
Virginia	5	1	0	1
Indiana	8, 9	2	0	2
Michigan	10	1	0	1
Louisiana	11, 12, 45, 46	4	0	4
Texas	13*, 14, 15, 16, 17, 18	5	1	6
Florida	19, 28*	1	1	2
Pennsylvania	20, 21*, 29, 30	3	1	4
New York	26*	0	1	1
	TOTALS	38	10	48

*Piperidine decomposing organisms lacking.

This means that slightly more than 21% of the tested soils lacked while slightly less than 79% possessed organisms which decompose piperidine.

4. BENZIDINE

According to Schreiner, Reed and Skinner (20) benzidine is toxic to crop plants in solution cultures. The question has been raised as to whether it persists once it gets into the soil, or whether it is destroyed by chemical or biological agencies.

A preliminary test indicated that benzidine served as the source of carbon as well as the source of nitrogen for the organisms under investigation and that their growth occurred more readily in solutions of low acidity.

To determine whether it is decomposed by microorganisms, cultures were made in the usual manner except that the solution had a reaction of 0.6% acid normal to phenolphthalein and contained benzidine. After incubating at room temperature for thirty days each culture was tested for the presence of benzidine by the benzidine blood test. A part of these results is shown in Table XXVII.

TABLE XXVII.—*Decomposition of Benzidine by Soil Organisms*

Soil	Bacteria	Molds	Benzidine
1	yes	yes	yes
2	yes	yes	no
3	yes	yes	yes
4	no	yes	no
5	yes	yes	no
8	no	yes	no
11	no	yes	no
13	yes	no	no
14	yes	yes	yes
15	no	yes	yes
16	no	yes	yes
18	yes	yes	no
19	yes	yes	no

From these results it appears that about half of the soils listed possess organisms able to decompose benzidine in solution cultures. Other experiments show that of the 45 soils tested seven contain organisms able to decompose it.

Distribution of Benzidine Decomposing Organisms in Several States

The accumulated data on the decomposition of benzidine soil organisms are given in Table XXVIII.

TABLE XXVIII.—*Benzidine Decomposing Organisms in Several States*

Source	Soils	Decomposed	Not decomposed	Total
Alabama	1, 2, 3*, 4, 6*, 7,22*,23*,24*, 25*, 27*, 31*, 32*, 33*, 34*, 35*, 36*, 37*, 38*, 39*, 40*, 41*,42*,43*,129*,130*	4	22	26
Virginia	5*	0	1	1
Indiana	8*, 9*	0	2	2
Michigan	10*	0	1	1
Louisiana	11, 12*	1	1	2
Texas	13*, 14*, 15*, 16*, 17*, 18	1	5	6
Florida	19, 28*	1	1	2
Pennsylvania	20*, 21*, 29*, 30*	0	4	4
New York	26*	0	1	1
	TOTALS	7	38	45

*Benzidine decomposing organisms lacking.

Summarizing these results it appears that seven, or 15.5%, of the 45 soils examined contained these organisms, while 38, or 84.4%, lacked benzidine decomposing organisms.

IV. DISCUSSION

There are more or less conflicting claims concerning the toxicity of certain organic substances in soils and in solution cultures. Schreiner, Reed and Skinner (20) who changed their solutions frequently to inhibit the growth of microorganisms hold that certain substances are injurious to crop plants in solution cultures; while Fraps (3), and Funchess (4) who made no attempt to inhibit the activities of such organisms in their soils contend that these substances are not toxic in most soil cultures.

The results of the present investigations place the emphasis not on the kind of substratum but rather on the factors which determine the persistence or the disappearance of toxins in the substratum. These factors are, primarily, the presence of organisms able to decompose the toxins, and, secondarily, favorable conditions especially of reaction and nutrient salts for the growth of the organisms. Hence, the frequent absence of harmful effects on seedlings grown in soils may well be referred to the presence in the soils of microorganisms able, under favorable conditions, to decompose the toxins, while the injurious effects of toxins on seedlings grown in water or solution cultures are probably due to the absence of such organisms or to some condition hindering their activity.

The data of these studies have some bearing on the persistence of toxins that might enter the soil. They indicate that the organisms which decompose certain toxins occur in many soils, that the organisms which decompose other toxins occur in but few, while those which decompose still other toxins do not exist in any soils tested. This being the case a toxin like resorcinol, the organisms of which occur commonly, may remain in the soil such a short time that it would seldom be found. In the case of salicylic aldehyde, which has not been decomposed by the organisms of any soil (7), the relations are very different. This toxin may occasionally be decomposed by chemical agencies in the soil. However, once it enters the soil it usually remains, hence the chances of its being found are nearly in proportion to the number of avenues through which it may enter.

In the light of these studies toxins may be expected to persist in soils when one or more of these conditions prevail, namely, unfavorable reaction, unsuitable nutrients, inadequate aeration, or absence of decomposing organisms. Presumably the toxins that have been reported as occurring in soils entered as constituents or as decomposition products of plant materials, and remained because bacteria or molds were either inactive or absent.

It is difficult to estimate the chances of a toxin persisting in a soil since the data obtained are mostly on the presence of decomposing organisms. Besides the occurrence of the organisms the influence of several conditions must be included in the estimate. From the data reported it may be estimated that cumarin may persist in 1 of 20 soils, while benzidine may persist in 6 out of 7 soils. It should be recognized that there is a vast difference between the occurrence of toxins in soils and their persistence once they find entrance.

Wheat straw, timothy hay, and alfalfa hay, which frequently find their way into soils, have recently been reported as containing toxins. Collison and Conn (1) have found vanillin and dihydroxystearic acid in wheat straw; dihydroxystearic acid in timothy hay; and vanillin and salicylic acid in alfalfa hay. They have shown that extracts of these plant materials cause stunting of barley seedlings; and that wheat straw extracts were very harmful to seedlings of barley, corn, and garden beans and less harmful to seedlings of garden peas and soy beans.

These results indicate an important means by which such toxins may find their way into the soil, and suggest a narrow gap between toxins in plants and toxins in soils as reported by Shorey (23). But, if the common occurrence and wide distribution of organisms able to decompose certain toxins is admitted, the small chance of finding these toxins in soils be-

comes apparent. Indeed, we need not expect to find the toxins in fertile well aerated soils of low acidity except in the few cases where the proper organisms are lacking. On the other hand there are toxin decomposing organisms so limited in distribution that they can hardly occur wherever the toxin is likely to be found in the soil. For example, hydroquinone is decomposed by the organisms of but one third of the soils tested, while salicylic aldehyde is decomposed by organisms of none of the soils tested even though many were fertile.

While none of the toxins used was decomposed by the organisms of all of the soils tested, the frequency of decomposing organisms gives ground for the belief that the majority of toxins are effectively destroyed by organisms rather than by chemical agencies, even though the chemical agency may be the controlling factor in some instances.

Therefore, unless the organisms are absent, the toxins distinctly antiseptic, or the conditions continuously unfavorable, we may expect the injuring substance eventually to be decomposed.

V. SUMMARY

1. The addition of calcium sulphate improves Robbins' solution for the growth of soil organisms.

2. Solutions but slightly acid to phenolphthalein yield the most abundant growth of a majority of soil organisms.

3. Seven toxins have been added to the list of those decomposed by soil organisms, namely, resorcinol, cinnamic acid, quinone, hydroquinone, caffein, piperidine, and benzidine.

4. Vanillin, cumarin, and resorcinol decomposing organisms are very common and widely distributed.

5. Cinnamic acid, caffein, and quinone are decomposed by the organisms of about two thirds of the widely distributed soils tested.

6. Hydroquinone is decomposed by about one third of the widely distributed soils examined.

7. About three fourths of the soils from the several states contain organisms able to decompose pyridine and piperidine.

8. A few soils contain benzidine decomposing organisms.

9. Vanillin, cumarin, cinnamic acid, and resorcinol decomposing organisms occur in one or more soils from each of nine states.

10. In order of mention, quinone, hydroquinone, caffein, pyridine, piperidine, and benzidine decomposing organisms occur in a decreasing number of states.

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