

souring but favored the development of an offensive, putrid odor, and a bitter taste. This effect was much less noticeable in the samples pasteurized at 65° than in those of the same origin pasteurized at 85°, the same precautions having been taken to prevent subsequent contamination in either case.

NEW YORK CITY, June, 1908.

THE ISOLATION OF PICOLINE CARBOXYLIC ACID FROM SOILS AND ITS RELATION TO SOIL FERTILITY.¹

BY OSWALD SCHREINER AND EDMUND C. SHOREY.

Received June 4, 1908.

Recent investigations have shown, contrary to the generally accepted belief since Liebig's time, that lack of productivity in soils is not always due to the lack of nutrient substances, but even with an abundance of these, lack of productivity may be produced by the presence of substances actually deleterious to plant growth. The presence of toxic substances has been virtually established by the properties which the soil and soil extracts possess. When an unproductive soil is extracted with water, the aqueous solution has been found to possess the properties of the soil itself so far as plant growth is concerned. When a good soil is extracted, a good soil extract is obtained, and a poor soil yields a poor soil extract, although the extract may have been made from adjoining fields of the same soil type and be practically identical in mineral composition. Such poor soil extracts produce in many instances plants which are far less developed than similar plants grown in pure distilled water under identical conditions. It has been found that these toxic properties can be removed from such a soil extract by a number of quite simple operations, such as treatment with carbon black, ferric hydroxide, and other absorbent agents, whereby the toxic material is apparently removed from the solution. This action seems to be quite common for all toxic soils studied although in other respects the properties of toxic soil extracts differ appreciably. In some cases the toxicity can be destroyed by merely boiling the extract and this is due in some cases to a destruction and in others to a volatilization. In the latter case the toxic properties can be found in the distillate. The toxicity can also be much weakened by dilution with pure distilled water so that in the weaker solution there may even result a stimulation in growth such as is commonly observed with a large number of poisons in weak solutions. These toxic properties are also overcome in many instances by the addition of comparatively minute amounts of pyrogallol, naphthylamine, and other compounds, a phenomenon which can be explained only by assuming that these compounds have acted upon the toxic substance, or have so altered the plant by stimulation

¹ Published by permission of the Secretary of Agriculture.

or otherwise as to overcome them. That mineral salts such as sodium nitrate or calcium carbonate also aid in the destruction of toxic substances has already been shown in a previous paper.¹

It is of course well known that toxic soil conditions can be brought about by excess of salts or the presence of unfavorable inorganic compounds, such as ferrous iron, etc., but the properties of the toxic soil solutions that have been examined in these laboratories point strongly to the fact that the toxic substances are organic and that the productivity of a soil depends largely upon the condition of the organic matter in the soil and the processes which are at work in destroying the plant remains.

The review just given has shown that the toxic properties of soils have been demonstrated and that the existence of toxic substances is a reality with which it is necessary to deal in future soil studies on the fertility and infertility of our agricultural lands. A very important phase of work in these soil studies remained to be done before a thorough knowledge of these substances and the best means of treatment in practice could be attained. Many of their properties it has been shown had already been determined in so far as they related to plant growth. Not only had their physical properties been studied, but the physiological properties, in their effect on the nutrition of plants or other physiological functions had also been ascertained for many soils. It remained to obtain some knowledge of the chemical nature and properties of these toxic substances, that there may be an intelligent treatment of soils containing them, treatment that will bring about certain destruction or change to non-toxic substances, or prevent their formation, instead of the rule-of-thumb methods now in use. Obviously one way to attain this knowledge is to isolate these substances from soils and study their properties.

The problem of isolating organic substances from soils has been attempted from time to time in the history of agricultural science. Braconnot,² Walser,³ Gyde,⁴ and others even attempted to show by chemical means the presence of the toxic substances and it is largely due to their failure to obtain positive reactions that the matter was dropped for many years. Investigations have been reported from time to time on the isolation of various humus acids, ulmic, geic, crenic, apocrenic, etc., but the composition of the acid isolated has been a most variable one differing with each investigator and with each method employed so that it would not be far from the truth to say that none of these acids were shown to exist and that we know almost nothing about their chemical composition or constitution. The work of isolating and studying the nature of such compounds

¹ Schreiner and Reed, *THIS JOURNAL*, 30, 85 (1908)

² *Ann. chim. phys.*, 72, 27 (1836)

³ *Untersuchungen über die Wurzelanscheidungen*, Tübingen, 1838.

⁴ *Trans. Highland and Agr. Soc.*, 1845-7, p. 273.



Fig. 1.

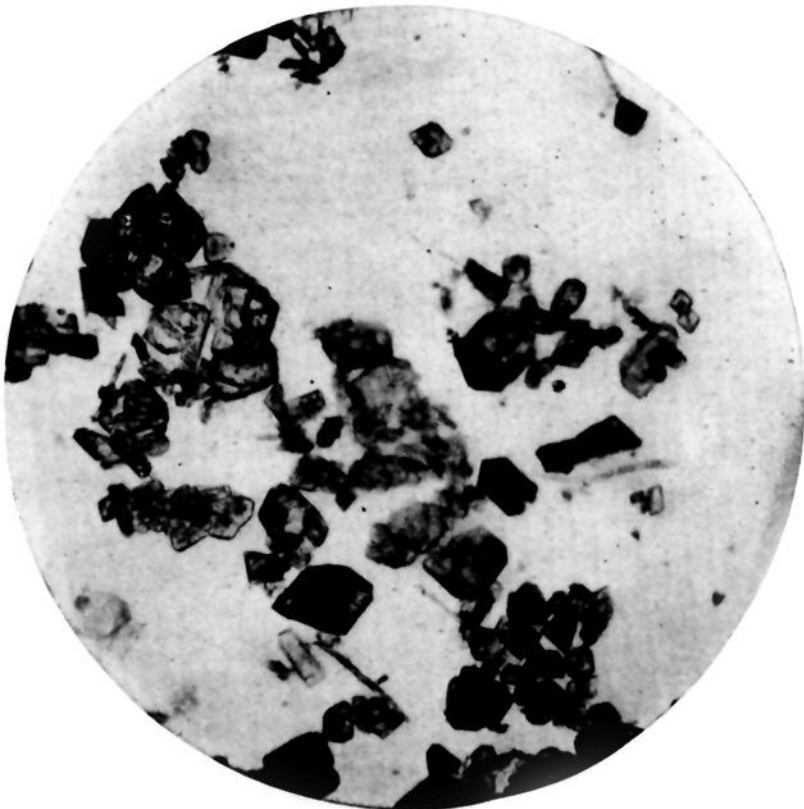


Fig. 2.

is necessarily slow and difficult to accomplish. In the first place, the amount may be very small and the composition of the organic matter of the soil is undoubtedly very complex, being made up of large numbers of organic chemical individuals. The absorption of the soil itself for organic substances is very great so that the ordinary solvents such as water, alcohol, chloroform, etc., take up but very minute amounts of organic material.

Then, too, there is an astounding lack of method for handling problems of this kind as there is no previous work which can serve in any measure as a guide. As pointed out above, it is questionable whether any pure organic compound has ever been isolated from a soil and identified until very recently, although it is obvious that a large number of organic compounds must exist. The amount of organic matter in ordinary soils is really very large, the average content being as high as 2.06 per cent. for the soil and 0.83 per cent. for the subsoil, determined on thousands of samples of soil and subsoil from all parts of the United States covering in all 237 types of soil. It is obvious that this organic matter is of very complex composition as its properties are quite different in soils from different localities. The amount of nitrogen also is considerable, as much as 0.1 to 1.0 per cent., and when we consider that only a small amount of this nitrogen is in the form of ammonia or nitrate and that the larger amount is in organic form, it is obvious that the problem becomes an important and very complex one.

It will be apparent to any one who realizes the paucity of knowledge of the organic matter in soils that the isolation of any definite organic compounds from the soil is not any easy task. When this is narrowed down to the isolation of toxic compounds the difficulties are greatly increased. Before outlining the methods by which this problem has been approached some of these difficulties should be considered for the matter resolves itself into a choice of methods to surmount these difficulties.

The water extract of an infertile soil usually contains but a very small quantity of organic matter, and it is a reasonable assumption that the toxic organic matter is only a part of the total organic matter present. Such being the case it is evident that the isolation of any organic compound from a water extract of a soil in sufficient amount to establish its identity and study its properties is in most cases a problem that does not come within the limitations of laboratory work. In other words, the amount of soil or soil extract necessary to handle would be too great for ordinary laboratory appliances and methods. This difficulty due to the small quantity of organic toxic material which must be present in the soil solution is much increased by the fact that the toxic substances are unknown compounds with unknown properties. The only certainty in modern toxicology in the detection of poisons in mixtures of organic matter such

as the organs of a dead body lies in the knowledge of the properties of poisons and their characteristic reactions. When, as sometimes happens, death is caused by a poison unknown or little known to science, little or no additional information is gained by chemical examination, and such an investigation, as to the cause of a death with symptoms of poisoning, generally ends without adding any definite chemical information. In many respects the isolation of toxic material from an infertile soil and the detection in the animal organs of a poison of which the identity, properties, characteristic reactions and symptoms produced are all unknown, are analogous.

With the water extract of a soil practically unavailable as a source of the unknown toxic substances under the present limitations of laboratory work, and lack of methods applicable to such research, the only course open is to make use of all other methods which are known or can be devised for effecting a solution of the soil organic matter, and then to attempt to isolate from such solution definite compounds, taking advantage of proven methods in organic and biological chemistry or devising new ones.

There are a few facts regarding the soil organic matter which are true more or less for any soil and which should be kept in mind throughout the discussion of the subject. The organic matter in soils is in general but slightly soluble in the ordinary neutral solvents, such as alcohol, ether or chloroform. Dilute acids at ordinary temperatures have also a little solvent action on soil organic matter and if heated there is usually change effected in the constitution of some of the organic compounds. Dilute alkalies at the ordinary temperatures, dissolve relatively large amounts of soil organic matter, in fact, in the case of some soils nearly the whole of the organic matter can be so dissolved by repeated treatment. This treatment of soils with dilute alkalies although it may in some cases effect some change in the constitution of some of the organic compounds, especially those nitrogenous, seems at present the only method of effecting the solution of a large part of the organic matter with the minimum chance of changing the character of the compounds sought and as such was the first method used in the attempt to isolate toxic organic compounds from the soil.

The soil examined in this way was from Takoma Park, Md. This soil which is characterized physically by large percentages of clay, silt and coarse sand, and very little of the intermediate material, is quite light in color and contains, contrary to what might be expected from its appearance, 3 per cent. organic matter. It contains 0.1 per cent. nitrogen and analyses of both water and acid solutions indicate the presence of sufficient mineral plant food for ordinary crops. It is, however, an exceedingly infertile soil and does not respond readily to treatment with either stable manure or commercial fertilizers. The water extract of

this soil is a poor medium for the growth of wheat seedlings, but is much improved by treatment with carbon black, dilution, addition of pyrogallol or calcium carbonate. In other words field observations of this soil indicate that something, other than lack of plant food, is the cause of infertility, and laboratory observations confirm this and in addition indicate that this infertility is due to the presence of some toxic substance.

This soil when treated with a two per cent. solution of sodium hydroxide gives a dark colored solution, the so-called humus extract. This solution, after separation from the soil residue and treatment with an excess of acid gives a flocculent dark colored precipitate, the so-called humic acid. The filtrate from this precipitate is dark colored but much lighter than the original extract and still contains a portion of the organic matter. Neutralization of this filtrate by the addition of sodium hydroxide produces a further precipitate containing both organic and mineral matter. The neutral filtrate from this is still colored dark yellow and contains a considerable portion of the organic matter originally extracted from the soil by sodium hydroxide. By dissolving both of the above precipitates again in sodium hydroxide and repeating the operations of acidifying, filtering, neutralizing, and again filtering, there can be obtained a still further quantity of organic matter in neutral solution, which can be mixed with the first portion. This process results in a neutral solution containing some of the organic matter and a comparatively large amount of a sodium salt resulting from the neutralization of the sodium hydroxide used.

This neutral solution of soil organic matter gives precipitates upon the addition of a number of mineral salts such as basic lead acetate, cupric acetate, mercuric nitrate, and silver nitrate, precipitates which contain organic matter and are usually nitrogenous.

The amount of soil organic matter in this neutral solution seems to be approximately the same whether hydrochloric, sulphuric, or nitric acid be used for neutralizing; acetic acid, however, usually gives a smaller amount in solution than the mineral acids. The final treatment or precipitant to which the solution is to be subjected must decide what acid is used throughout, *e. g.*, hydrochloric acid would not be used if silver nitrate was to be the precipitant, nor sulphuric acid if basic lead acetate was to be used.

The method finally adopted for isolating a definite crystalline compound was to treat the soil for twenty-four hours with frequent shaking with a 2 per cent. solution of sodium hydroxide free from chlorides and allow the mixture to stand until the soil residue settled when the dark colored solution was syphoned off. To this was added a slight excess of nitric acid free from the lower oxides of nitrogen and the flocculent humus precipitate filtered off. The acid filtrate was then exactly neutralized by the addition of sodium hydroxide and the precipitate so formed filtered

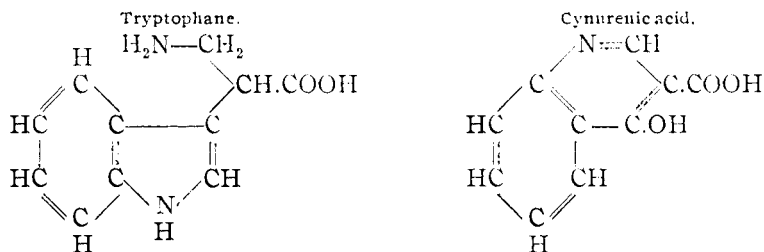
off. To the resulting neutral solution was added a solution of silver nitrate and the precipitate formed, which was slight in amount, was allowed to settle. The clear liquor was decanted and the precipitate collected on a small filter and washed with a little water. The precipitate while still moist was suspended in water and hydrogen sulphide passed through until decomposition was complete. The silver sulphide was filtered off and the filtrate evaporated to small volume on a water bath and allowed to cool slowly. There was thus obtained a more or less colored residue from which crystals formed. The crystals were separated from the mother liquor, washed with a small quantity of cold water, dissolved in hot water and precipitated again with silver nitrate. Filtration, treatment with hydrogen sulphide, etc., being now repeated, the crystals were usually obtained fairly free from color and could be further purified by recrystallization from water.

The crystals thus obtained are slightly soluble in cold water, easily in hot, slightly soluble in alcohol and almost insoluble in ether. The compound crystallizes from water in oblique prisms with water of crystallization which is lost at 100°. In Fig. 1 is shown a microphotograph of these crystals obtained by allowing a rather dilute solution in hot water to cool slowly, and Fig. 2 shows the crystals obtained by cooling a rather concentrated solution in water. The latter crystals are usually very thin plates, superimposed, forming large scale-like masses, and giving very brilliant color effects with polarized light. It sublimes unchanged on heating in an open dish and does not melt in a capillary tube at 300°. A water solution is faintly acid to litmus and when neutralized does not give a precipitate with either barium chloride, calcium chloride, cadmium sulphate, or lead acetate. Cupric acetate gives a bluish crystalline precipitate insoluble in cold water. Silver nitrate gives a flocculent amorphous precipitate. The compound has therefore the appearance and properties of picoline carboxylic acid, a compound already obtained by Shorey¹ from an Hawaiian soil by the same method. The compound from Hawaiian soil was further identified as picoline carboxylic acid by its corresponding water of crystallization, its nitrogen content, the chloride content of the hydrochloride and its conversion into lutidinic acid on oxidation. The last-named acid is characterized by crystallizing in leaflets slightly soluble in cold water, melting at 239°, and giving a yellowish orange color with ferrous sulphate.

The preparation obtained from the Takoma soil having the properties stated above was compared with that obtained from the Hawaiian soil and also with picoline carboxylic acid prepared artificially in the laboratory and found to correspond throughout.

Picoline carboxylic acid, which has heretofore been known as a labora-

¹ *Report of Hawaii Agr. Expt. Station, 1906, 37.*



which, as is readily seen, contains the possibility of the pyridine closure as illustrated by its transformation into cynurenic acid. All these facts seem to indicate strongly that the origin of the pyridine ring in the soil, whether it be as picoline carboxylic acid or other pyridine derivations, lies most probably in the dissociation of proteins.

As mentioned above, there has been gathered some evidence of the presence of pyruvic acid in the Takoma soil, but this is as yet indicative only. The evidence stated briefly, is as follows: When the neutral soil extract obtained, as outlined above, is made alkaline with sodium hydroxide and then a solution of iodine in potassium iodide added, there is formed in a short time, at room temperature, a precipitate of iodoform. The same precipitate can also be obtained by treating the original sodium hydroxide extract of the soil with iodine reagent. The iodoform so obtained was identified after recrystallizing by its melting point and by its characteristic crystalline form as well as its odor. Pyruvic acid forms iodoform when treated in this way, but this is true of other acids, for instance, laevulinic. Again, when the neutral soil extract is made acid and treated with a solution of phenylhydrazine hydrochloride there is produced in a short time a microcrystalline reddish colored precipitate, which tends soon to become resinous. It was not found possible by any means so far tried to purify this precipitate so as to obtain any characteristic crystalline form or constant melting point. The phenylhydrazone of pyruvic acid crystallizes in needles and melts at 178° to 183° . It is evident that if pyruvic acid is present there is also present another compound which likewise gives a precipitate with phenylhydrazine. Bülow has described a reaction of the phenylhydrazones, which he states is characteristic. When the phenylhydrazone is treated with concentrated sulphuric acid and potassium dichromate there is produced a purplish color. This color is produced by the phenylhydrazone precipitate from the soil extract and by the pure phenylhydrazone of pyruvic acid. Simon² has described a color reaction of pyruvic acid, which he states is characteristic. This depends on the blue color given by sodium nitroprusside and ammonia when added to a dilute solution

¹ *Ann. Chem.*, 236, 195 (1886).

² *Compt. rend.*, 125, 534 (1867).

of pyruvic acid. When the neutral soil extract is treated with these reagents there is produced a very slight greenish color which, since the soil extract is of an orange color, might be expected in the presence of small amounts of pyruvic acid. The delicacy of the reaction was tested by adding known quantities of pyruvic acid to the neutral soil extract and adding the reagents and it was found that the reaction was not at all delicate in the presence of the other material present in the soil extract, as much as 500 parts per million being required to give the characteristic blue color, lower concentrations producing greenish shades. These reactions indicate at least the probable presence of pyruvic acid as well as other compounds not yet isolated. The possible presence of this acid in the soil is of interest for it may be that through the agency of ammonia in the soil the change from the open chain non-nitrogenous pyruvic acid, to the ring form nitrogenous uvitonic acid may be effected as is done in the laboratory with alcoholic ammonia. Uvitonic acid has been sought for in the various soil extracts, but so far with no indication that it is present.

When picoline carboxylic acid is prepared from the neutral soil extract by the method stated above there is separated from the crystals a dark colored mother liquor containing nitrogen. When this is evaporated nearly to dryness there is usually a further separation of a small quantity of picoline carboxylic acid but no other crystalline substance has been obtained or observed. The mother liquor at this stage, however, is thick and viscous and of such a nature that crystallization of any substance present would be difficult. When the mother liquor from which picoline carboxylic acid has been separated is made alkaline with sodium carbonate and treated with a solution of diazo sulphanilic acid a dark red color is slowly formed. The color produced by this reagent with certain organic compounds was first noticed by Ehrlich¹ and further described by Pauly,² who found that it could be used for the detection of histidine. Histidine gives with this reagent a dark red color, which remains red on dilution and no other protein decomposition product except tyrosine gives this color, all others giving yellow colors, which disappear on dilution. According to Pauly, the distinctive color given by histidine and diazo sulphanilic acid is due to the imidoazole group, histidine being α -amino β -imidoazole propionic acid. The color obtained by treating this material with diazo sulphanilic acid does not appear to be that obtained with histidine, being slower in appearing and soon losing the red tint on dilution. The same color can be obtained in the distillate on distilling the original soil with steam and is much more pronounced if the soil be made slightly alkaline before distillation. It has been found

¹ *Z. klin. Med.*, **5**, 285 (1882).

² *Z. physiol. Chem.*, **42**, 508 (1904); **44**, 159 (1905).

that other organic compounds than those mentioned give this color, for instance, arbutin¹ and piperidine, so that the most that can be said of this reaction is that it indicates the presence in the soil of some substance not yet isolated or identified.

In the study of soil fertility from the point of view stated in the first part of this paper, the first and immediate interest concerning any organic compound isolated from or proven to be present in a soil is with regard to the possible toxicity of such compound to plants. While it is probably true that every organic compound in the soil which is at all soluble, is, to some extent, a factor influencing plant growth, either favorably or unfavorably, and that before there can be any true science of agriculture, the chemistry of the organic soil material, non-toxic as well as toxic, must be known, it is evident that at this stage the interest of the investigator as well as of the practical agriculturist centers around the organic soil compounds which are toxic. Once the identity of a toxic soil compound is established, it can probably be prepared artificially in quantity sufficient for an extended study of its effects on plants, both alone and as modified by other soil compounds or added chemicals, fertilizers, etc., an investigation which cannot but furnish much of scientific interest and practical value.

Anything like complete knowledge of the organic matter of soils or even of one soil can be arrived at only after a long research—research in a field as yet almost unknown and one presenting many difficulties incident to ignorance of the matter under investigation and inadequacy of available methods. Such research will follow as the natural result of the demonstration of the presence of toxic organic compounds in soils in the most positive and practical manner—by isolating such compounds so that they can be seen, handled and experimented with.

In determining the toxicity of substances isolated from soils, wheat seedlings have been used as an indicator of toxicity in the manner described in detail elsewhere.²

Wheat seedlings three or four days old, of uniform growth, were grown in solutions of the substances tested, control cultures of similar seedlings in pure distilled water being carried on at the same time. The transpiration, green weight of plants at the end of the period of growth, generally ten or twelve days, together with observations made from day to day on the appearance of the roots and tops, furnished the data from which the conclusion as to toxicity or non-toxicity was drawn.

The method by which picoline carboxylic acid was isolated from soils was essentially the precipitation of the acid as either the lead or silver

¹ THIS JOURNAL, 30, 95 (1908).

² *Bull. Ins. No. 28, 36, 40, 47, Bureau of Soils, U. S. Dept. of Agr.*

salt from a neutral solution of a portion of the soil organic matter. The amount obtained from the soil by this method was very small. For this reason, and more particularly to meet the objection that any observed toxicity might be due to minute traces of lead or silver incident to the method of preparation, the toxicity of this compound was tested with the substance prepared artificially. The method of preparing this, which has already been stated, is in outline, the treatment of pyruvic acid in alcoholic solution with dry ammonia gas, precipitation of uvitonic acid with sulphuric acid and splitting of this into picoline carboxylic acid and carbon dioxide on heating. The yield, however, is poor.

The following figures give the results obtained with wheat seedlings grown for ten days in solution of different strengths of artificial picoline carboxylic acid.

TABLE I.—EFFECT OF PICOLINE CARBOXYLIC ACID ON WHEAT SEEDLINGS.

| | | Relative transpiration. | Relative green weight. |
|-------------------------------|-------------------------|----------------------------|---------------------------|
| Control in distilled water | | 100 | 100 |
| Picoline carboxylic acid..... | 1 part per million | 140 | 95 |
| " " " " " " " " " " " " | 10 " " " " " " " " " " | 105 | 101 |
| " " " " " " " " " " " " | 50 " " " " " " " " " " | 107 | 98 |
| " " " " " " " " " " " " | 100 " " " " " " " " " " | 85 | 89 |
| " " " " " " " " " " " " | 200 " " " " " " " " " " | 55 | 70 |

It is seen from the figures above, substantiated by the results of other series, that this soil constituent is toxic to wheat seedlings and in low concentrations is a stimulant, an effect which is, however, characteristic of small doses of poisons when applied to plants. In the solutions containing 100 and 200 parts per million, in addition to the decreased transpiration and green weight of plants, the roots showed an abnormal appearance, stunted growth with root tips first darkened, then club shaped, or hooked, characteristic of the presence of a toxic compound. However, since this compound does not show marked properties in solutions containing 50 parts per million and, so far as can be determined, occurs in less amount in the toxic aqueous soil extract from the soil examined, it does not seem likely to explain by its presence the full effect of the toxic properties of the extract from the soil in which it was found. While this toxicity, as will be shown in another paper, is undoubtedly due to the presence of the more toxic dihydroxystearic acid in this particular soil, nevertheless, it was thought to be of interest to study in this regard several compounds closely related to the picoline carboxylic acid.

Uvitonic acid, from which picoline carboxylic acid is formed simply by heating to the melting point, and which differs from it only by carbon dioxide, is so closely related that it was thought desirable to test its toxicity even though there was no evidence of its presence in the soil.

The result of such a test with wheat seedlings grown for twelve days in solutions of different strengths of uvitonic acid is shown in Table 2:

TABLE 2.—EFFECT OF UVITONIC ACID ON WHEAT SEEDLINGS.

| | | Relative transpiration. | Relative green weight. |
|----------------------------|--------------------|----------------------------|---------------------------|
| Control in distilled water | | 100 | 100 |
| Uvitonic acid..... | 1 part per million | 95 | 107 |
| " "..... | 5 " " " | 88 | 116 |
| " "..... | 10 " " " | 87 | 108 |
| " "..... | 50 " " " | 39 | 78 |
| " "..... | 100 " " " | 20 | 68 |
| " "..... | 200 " " " | 35 | dead |

The plants growing in concentrations of 200 parts per million had been dead two days when the test was terminated and the transpiration of this set is higher than the preceding set, owing to this death of the plants, as dying plants frequently show increased transpiration.

Uvitonic acid, it is evident, is quite toxic to wheat seedlings, much more so than the nearly related picoline carboxylic acid. It is also reported to be strongly antiseptic, which is again an indication of strong toxic properties for higher plants.

The relative toxicity of these two compounds brings up the question of the relation of toxicity to chemical constitution. Uvitonic acid and picoline carboxylic acid are related, as shown by the structural formulas already given, the former differing from the latter by carbon dioxide, which, on heating, splits off from the carboxyl. Uvitonic acid is a dicarboxylic acid, and the greater toxicity would seem to be associated with the presence of the second carboxyl group. However, all the carboxylic acids of pyridine and its homologues having one, two, and three carboxyls are very weak acids. They form salts with bases in the usual way, but have also the power of forming salts with mineral acids by direct addition, for instance, the hydrochloride, by the direct addition of hydrochloric acid. It is only in the higher members of this group, for instance, where all the hydrogen atoms are replaced by carboxyl, that the acid properties predominate and the power to form salts with mineral acids is lost. The whole question of the relation of toxicity to chemical constitution is one for future investigation, the data at present available furnishing little more than suggestions of possible explanations.

Pyruvic acid was found to be quite toxic to wheat seedlings, but was not so in the form of its sodium salt and its activity as a factor in fertility, if present in the soil, would no doubt depend on the form in which it occurred.

Other related compounds, which may have a possible soil interest in this connection, have been studied for toxicity with the following results:

Pyridine (C_5H_5N) was found by Laurent¹ to be toxic in a one per cent. solution to the fungi which he studied. It has been observed by Falkenburg² that the vapor of pyridine and some of its homologues is poisonous to bacteria.

In the experiments with wheat seedlings by Schreiner and Reed³ it was found that pyridine, although not sufficiently toxic at a concentration of 1,000 parts per million to kill the wheat plants in nine days, was, nevertheless, very injurious, especially to the growth of the green parts of the plants. In a concentration as low as 50 parts per million the growth of the tops was inhibited and the leaf tips turned brown. In the lower concentrations there was no stimulation of growth.

Picoline ($C_5H_4N.CH_3$), or methyl pyridine, was toxic to wheat seedlings, but only killed in the concentrations of 1,000 parts per million, and did not cause injury below 500 parts per million. The injury seemed to be manifested by the tops more than the roots, thus resembling the action of pyridine.

Piperidine ($C_5H_{11}N$), or hexahydropyridine, killed and injured at a lower concentration than either pyridine or picoline. A sample of piperidine that was neutralized with acetic acid proved to be more toxic than the strongly alkaline piperidine itself. Piperidine seems to injure the roots more severely than the tops.

While picoline carboxylic acid may not be an important factor in the infertility of the soil studied, the fact that it has been found in other soils in larger quantities, its moderate toxicity, at least so far as wheat seedlings are concerned (although it is not impossible that toward other plants the compound may be either more toxic or less so) and its close relationship to other pyridine compounds of greater toxicity places it among organic soil toxins to be considered and sought for in the general study of unproductive soils.

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A CHEMICAL EXAMINATION AND CALORIMETRIC TEST OF INDIANA PEATS.

By R. E. LYONS AND C. C. CARPENTER.

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The lake region of northern Indiana, which is embraced in the three or four northern tiers of counties, contains hundreds of thousands of acres of peat bog. Twenty-nine representative samples of peat from this territory, obtained through the Department of Geology and Natural Resources of

¹ *Ann. Soc. Belge. Micr.*, 14, 29 (1890).

² Czapek, *Biochemie der Pflanzen*, II, 926.

³ *Bot. Gazz.*, 45, 73 (1908).