The Effect of Various Organic Compounds on

The Growth of Azotobacter.

Major Thesis

in

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Submitted by

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The intricate problem of soil fertility, its underlying causes, its promotion and its conservation - these questions have for the past century been the dominating phase of agricultural research. The complexity of the problem has attracted an astonishing number and variety of workers; scarcely any related branches of science have failed to contribute something towards its understanding. Foremost in the field was the chemist, backed by the force of his analysis which showed an apparently plain relationship - the invariable presence in the plant of certain elements, the usual abundance of these elements in the soil, a condition simple enough regarded solely from these established and somewhat correlated facts. Later, the work of the physicists indicated other important factors which influenced soil conditions and in succession new and commanding ideas have introduced themselves into the subject. Recently, the science of bacteriology has explained certain fundamental processes which take place in the soil and vitally effect plant growth, processes which are inseparately related to the conception of "available" plant food, and the explanation of which has led to a more rational treatment of soils than innumerable chemical analyses could do. Physiologists also, are busy interpreting facts which they regard as significant, and these will perhaps later assume conspicuous places in a generalized consideration of the subject.

Naturally each group of workers has brought forward its favorite theory on the relationship of the soil and the plant, emphasized it themselves, and had it received by others with the qualifications usually accorded new ideas. Which of these theories to regard as most logical in its interpretation, which in practice has proved most satisfactory by results, is a question yet unsettled. But of all these ideas perhaps hone has been so persistent as the conception of necessary additions of mineral food to the plant - the "plant food" theory of fertilizers. Receiving as it did enunciation from the celebrated Leibig and confirmation so far as chemical analysis can confirm, the force of the idea in explaining soil fertility has constantly grown, perhaps too forceful and relegated others to places of secondary consideration. If it be accepted as foremost in influencing crop production, then as an agricultural policy, it is singularly ill adjusted. The very variability in the application of mineral fertilizers is testimony of this. Advocates of "~~mixed~~ complete" and those of "incomplete" fertilizers are separated by rather irreconcilable differences, and such widely different viewpoints suggest skepticism on the validity of the whole idea - or at least on the emphatic importance usually accorded it.

But the present status of the question of soil fertility is an exceedingly hopeful one. It is not one of narrow and dogmatic opinions, of intolerant exclusion of new ideas ans explanations. Bacteriology has proved its usefulness and other phases of science are doing likewise. If, indeed, the whole question be as yet poorly explained, it is not one to which access with a new thought is difficult, even if the idea be in ill accord with prevailing and accepted ones.

There is another theory to account for the unproductivity of soils, a conception which may be termed biochemical and which would emphasize the organic rather than the inorganic status of the soil as most vitally affecting plant growth. This idea, while accepted by few as comprehensive enough to explain the major difficulties of the question, and regarded by others as of little or no import, is yet constantly asserting itself with increasing and fascinating interest. The great majority of soils contain organic substances which are decidedly deleterious to plant growth. Furthermore, it seems well established that the more unproductive soils contain these toxic substances in larger quantities and more varied forms than do fertile soils. It is the extension of and inference from these statements upon which the whole theory rests. Grouping them together and with them the almost compelling fact of the abundance of mineral food in most soils, one has a theory which is calculated to recur and to incite interest. Evidence pointing to the reasonableness of the idea is by no means wanting. True, some of it is of a rather speculative character, but other is ~~upheld~~ supported by careful and painstaking experimental proof. The extent and quality of that which has been already offered certainly warrants consideration of established facts and would, it seems, inspire further research on the subject.

A complete review of the investigations up to the present century which were directed towards a study of the toxic conditions of the soil would be exceedingly brief. The work consists chiefly of some observations on the excretions of seeds during the process of germination and on substance exuded by growing plants. The most extensive work of this nature was that done by Macaire[a] who reported, through some rather empirical experiments, that plants exude substances during this period of growth and these substances are usually toxic to further growth of the plant. He cites the work of Brugman[b] as confirmatory to his own. Boussingault[c] reported a similar study but with the conclusion that only in water cultures does the exudation take place.

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a. Macaire. Prinsep, Memoire pour servir a l'histoire des assolemens; mem de la soc de physique et l'histoire nat. de Geneve. 5,282-302.(1832)

b. Brugman, De mutata humorum in regus organico indole. 1786.

c. Boussingault, Jean B.J.D., Rural Economy. Trans. by Geo. Law. London. 1045. p. 345.

Yet the famous French physiologist, De Candolle, accepted the work of Macaire and Brugman, and associating this with his own reasoning and knowledge on growth relationships, deemed it sufficient evidence upon which to base a theory to explain the value of crop rotation. (Physiologic Vegetable, p 1499). He reasoned, obviously, that the continuous cropping of land to the same species of plant resulted finally in a condition of toxicity from the exudations of the plant itself - certainly a reasonable analogy from related physiological facts. The introduction of plants of a widely different character into the soil was not accompanied by these toxic effects since each species of plant was affected only by its own exudations. The undeniable value of crop rotation, the "sickness" of soils when grown continuously to certain crops - it is doubtful if these conditions have been more satisfactorily explained than by this idea of De Candolle's.

By far the most notable contribution to the knowledge of the whole subject has been made within the past decade. It has come chiefly from one source, the Bureau of Soils of the U. S. Department of Agriculture, and has been of such nature as to receive general reference as the "Toxic Theory of the Bureau of Soils". These researches, classical certainly from the standpoint of biochemistry, have revealed some interesting possibilities regarding the effect of organic soil constituents on plant growth. They provoked possibly too sanguine conclusions on the part of the investigators in the earlier period of the study, yet this does not vitiate the legitimate merit of the research. It stands among the far reaching contributions to scientific agriculture of more recent years. The impulse to the investigation was furnished by the results of a study of a soil peculiarly notable for its unproductiveness. Attracted by this condition of singular infertility, and by other characteristics of the soil, uncorrelated to the one first mentioned, its normal texture, its average moisture content, the favorable comparison of its extract with that of fertile soils as regards mineral nutriment, the investigators pursued a synthetical method of examination rather than an analytical one. Summarizing their results in the initial publication[a] of the researches, the authors concluded that the soil contained some substance or substances probably organic in their nature which was toxic to the growth of wheat plants. The toxicity of the soil was corrected in either of two strikingly unrelated ways; by the use of stable manure, green manure, leaves of sumac or oak, tannic acid, pyrogallol, calcium carbonate, ferric hydrate, and carbon black. Tannic Acid and pyrogallol, it was concluded, probably acted chemically

a. Properties of an Unproductive Soil; Livingston, Britton, and Reid. U. S. Dept. Agr. Bureau of Soils. Bul.No.28. p. 39.

on the toxic substance while carbon black and ferric hydrate served undoubtedly as absorbents. The question of mineral nutriment was not considered a controling factor in the fertility of this particular soil. Such results were certainly indicative of inhibiting organic factors; they suggested that the so called "exhaustion" of soils might not be a depletion of mineral matter but an accumulation of organic poisons - the products of decomposing animal and vegetable matter and the excreta from growing plants. With such an idea in view, further extensive investigation was carried on by the Bureau of Soils. The inquiry was directed mainly along the following lines - a study of the extract of various soils, the growth of plants in them, with and without the addition of the substance known to emilorate the toxic condition; the theory of De Candolle was reviewed and extracts which had previously grown plants were investigated for toxic excreta; the effect on growing plants of organic compounds known to occur in the soil or in plants was noted; and finally, the organic compounds themselves were isolated from the soil and their properties studied. The observations reported in Bul.28 were apparently confirmed by further experiment. That the substance exerting the deleterious effect was organic seemed to be unquestionable and furthermore it could be removed by chemical or absorptive means. Perhaps more interesting still was the evidence[a] supporting the idea of toxic excretion of plants. Wheat seedlings grown in extract which had previously grown wheat exhibited the same signs of poisoning as did

a. Some Factors Influencing Soil Fertility. U. S. Dept. Agr. Bureau of Soils. Bulletin No. 40.

seedlings grown in the extract from an unfertile soil, yet when wheat was grown in the extract succeeding cowpeas there was little toxic effect noted. The toxic excreta from the plants was removed in the same manner as that in the original extract.

[a] A synthetical method was adopted for studying the effect of the organic soil constituents on plant growth. A number of compounds known to be derivative of animal and vegetable matter, and, therefore, likely to be present in the soil, were chosen for this work and their effect in various concentrations, noted on the growth of plants. The possible source of the toxic organic substance in the extract of unfertile soils was revealed in this study. Some of the compounds were toxic in extremely minute quantities and perhaps the majority were deleterious in concentrations in which they are likely to be present in the soil.

The phase of the entire research which was probably most fruitful in its results was that which concerned itself with the isolation and identification of the organic constituents of the soil. Some forty of these compounds have been isolated during the progress of the work which is yet under way and ~~it~~ which forms the only intelligent inquiry into the nature of soil "humus" which agricultural science has today. The compounds themselves have been studied with reference to their toxic properties, revealing in most instances inhibiting effect on plant growth. Whether their presence is a decisive factor in soil fertility is

a. Certain Organic Constituents of Soils in Relation to Soil Fertility. Schreiner, Reed and Skinner; U. S. Dept. Agr. Bureau of Soils. Bul. No. 47.

certainly unsettled but such a contribution to the knowledge of the constituents of soils is of vast importance.

A consideration of the work of the Bureau of Soils leaves little to report on experiments dealing with the idea of a toxic organic condition as a major factor in the question of soil fertility. A few isolated experiments have been run but none with the precision and care, with the elimination of limiting factors of experimental methods, that has characterized the Washington researches. It would seem, therefore, that whatever be the present viewpoint on the toxic theory of soils it must be colored by the conclusion from the work just cited with of course such indivicual interpretation and qualification of these results as each one may give to them.

The influence which the organic constituents of the soil may exert on its productivity has been studied heretofore with reference mainly to the growth of higher plants. That deleterious substances of an organic nature exist in many soils and their presence is the source of considerable toxicity to plants growing in such soils, is strongly indicated by previous work along this line. Investigations which have had as their controling idea an inquiry into the nature of their compounds, derived as they are either from soil humus or from the excreta of growing plants, have presented some rather strong evidence in support of the toxic theory of soil infertility. The nature of the results of these investigations would certainly warrant an extension of the research into other physiological fields.

The ~~plan~~ phase of study which would present the closest relationship to that already pursued and at the same time contribute something to the question of soil fertility, would concern itself with the bacterial flora of the soil. There is no more vital factor in the fertility of agricultural lands than those processes which are the result of microscopic plant life. The close relationship which has been shown to exist between these microscopic forms and the higher plants whose growth they so intimately effect, their response to the same medium of stimulation or depression, their close analogy in most physiological functions - these facts justify the conclusion that any abnormal condition affecting the one would have a corresponding effect on the other. If there are organic poisons in the soil which are toxic to growing plants, would not such fundamental processes as nitrification and nitrogen fixation reflect likewise the depression?

The theory which would account for the unproductiveness of soils by the presence in them of toxic substance assumes two possible sources of the inhibiting compounds - the vegetable or animal matter present in or applied to soils as the one, and the excretions of growing plants as the other. While, as previously noted, the effect of these organic poisons on the micro-organisms which influence soil fertility has been scarcely noted, yet, the bacteria as a division of plant life have received extensive study on the nature of their own excretions. In fact it is in this connection that some of our most important principles of immunity have been evolved and with them complete triumph over a vast number of diseases. So well established is this fact, the knowledge that bacterial life produce products poisonous to living forms of the same or related species that it scarcely seems necessary to review the literature in this connection. It must not be assumed, however, that such a fact is presented to corroborate an idea that the excreta of soil bacteria is a factor in soil fertility, but it does lend credence to the theory that higher plants, during this process of growth, excrete substances which accumulate in the soil to the detriment of succeeding crops.

In connection with some studies on free nitrogen fixation which are being pursued in these laboratories, studies on fixation by soils of various kinds when supplied with proper carbohydrates, and on the free nitrogen fixing organism, Azotobacter, its activity in pure cultures, and general physiological functions, the work reported in this paper was carried on. There are few biological processes worked out in the soil which are of more scientific interest than those which help maintain the nitrogen supply through the means of non-symbiotic, atmospheric fixation. Especially is this true of that aerobic group of organisms which were reported first by Beijerinck in 1901 and which are designated by the general term Azotobacter. Since their discovery at that time, this phase of soil bacteriology has received emphatic attention at the hand of many investigators. Nor have the researches failed to furnish results commensurate to the efforts expended on them. The widespread occurrence of the organism, the index which its presence and activity give to the fertility of a soil, are matters of no little scientific and practical interest. Only recently the author noted an increase of --15 milligrams of nitrogen per 10 grams of soil incubated in Ashby's solution, and a pure culture of the organism from another source, cited subsequently in this paper, gave a similar fixation in the same medium. Should an approach to these results be made possible under field conditions, it would certainly exert a profound influence on the maintenance of soil nitrogen. [a]The activity, in some sections, of the Azotobacter in accumulating atmospheric nitrogen has resulted, it is alleged, in such concentration of nitrates as to render the soil toxic to plant growth.

a. Wm. P. Headden. Colorado Agr. Exp. Station. Buls. No. 155,160,178

Indeed it is not unlikely, in the face of what is at present known of the organism's activity, that much of the organic nitrogen of soils must be referred to this and other closely related sources.

In regard to the possible effect of the organic constituents of the soil on the growth of Azotobacter, this group of organisms has shared the general inattention accorded most other bacteria. Their susceptibility to any kind of poisons has received only limited study. Experiments with carbon bisulfid[b], doubtless induced by the recent theory of its antiseptic action on soils, found that substance fatal to Azotobacter in concentration of 1.7 to 1.000. The food requirements of the organism, its most economic utilization of various carbohydrates has been more thoroughly investigated. Ashby in 1907

b. Maassen and Behn; Mitt. K. Biol. Anst. Land u. Forstw., 1907. No.4. Pp 38-42.

gave the formula for the media which bears his name and which is peculiarly adapted to the development of the organism. The superiority of mannite as a source of energy has been further demonstrated[a] as have many factors which promote the maximum efficiency in fixation. These are but examples of numerous studies along this phase to which the Azotobacter have been subjected.

The growth of Azotobacter can be directly measured by the increase of nitrogen in the medium in which it grows. This property of the organism makes it ideally suited to study the effect of various compounds on its development. Any stimulation or depression which might result, would be reflected in a total nitrogen analysis of the culture, especially if the result be compared with that from another culture grown in the same medium under identical conditions of incubation but without the addition of the compound in question.

It was in pursuance of this ~~method~~ principle that the studies herein reported were made. In the choice of organic compounds, the writers were directed chiefly to those which have already been studied with reference to their effect on the development of higher plants, many of which have been isolated from the soil, and are known to be likely constituents of various plants. The compounds were of the purest chemical nature obtainable, Merck's Blue Label reagents being used throughout the study. An effort was made, also, that they represent the various groups of organic substances likely to be present in the soil.

a. Hoffman and Hammer; Some Factors Concerned in the Fixation of Nitrogen by Azotobacter; Centralbl. f. Bact. Abt. 2. Bd. 28.1-127-1910.

Plan of the Experiment.

One litre Erlenmeyer flasks, to which were added 15 grams of pure sea sand, previously washed and burned, afforded an excellent surface upon which the Azotobacter developed. To each of these flasks, was added 100 c.c. of Ashby's media of the following composition:

Mannit	12	grams.
Mono-potassium phosphate	.2	"
Magnesium sulfate	.2	"
Sodium chlorid	.2	"
Calcium sulfate	.1	"
Calcium carbonate	5	"
Distilled water	1000	"

The flasks were then sterilized under 15 pounds of steam pressure for 15 minutes. After this sterilization, the compounds were introduced into the flasks in desired concentrations and all flasks received equal inoculation of pure cultures of Azotobacter previously grown on Ashby's Agar and suspended in sterile water. Two flasks were set up for each compound in every concentration, that duplication might be afforded and in many cases four and six flasks were finally run. Two control flasks receiving only inoculation were used to test the fixation power of the culture used. The experiment was run usually in sets of twenty flasks and all, including the control, were incubated for 21 days at the end of which time nitrogen determinations of the content of each flask were made by the Kjeldahl method.

The objection will be raised that since the compounds were added to the flasks after the latter had been sterilized a source of contamination was here made probable and the work cannot be regarded as a pure culture study. While this was true in some cases, the contamination was not of such nature as to vitiate the results in this particular work. It is extremely unlikely that any organisms introduced in this manner would effect nitrogen fixation. To test this point a series of flasks were set up with the compounds added after sterilization but receiving no inoculation. There were indeed, some which exhibited evidence of contamination yet none showed any perceptible gain in nitrogen. The same strain of Azotobacter was not used in every set of experiments since it is probable that this organisms loses some of its virulence when kept for some time under laboratory conditions. But all comparisons were made with the same culture, that is, the control represents the growth of the culture without the compound as compared to the corresponding strain with it.

Table I gives the effect of adding certain non-nitrogenous compounds in various concentrations to the culture neutrient. The results are those from analysis of each flask or the average of two which narrowly approached each other.

The Effect of Certain Organic Compounds in Various Concentrations on the Growth of Azotobacter.

Compound.	Concentration. p.p.Mil.	Milligrams of Nitrogen per 100 c.c. of Ashby Media.	Control.
Esculin	500	10.3	10.2
"	1000	11.3	8.4
"	2000	(14.4 (15.9	(9.8 (8.9
"	2000	(10.9 (9.5	(5.8 (5.
"	2000	(12.7 (11.6	10.1
Vanillin	500	(7 (7.8	10.2
"	1000	(2.9 (4.3	8.4
Daphnetin	500	13.44	(15.8 (14.2
"	1000	9.2	10.1
Cumarin	250	(5.5 (5.7	8.4
"	500	(8.8 (9.2	10.2
Pyrocatechin	250	(4.06 (5.	8.4
"	500	6.1	10.2
Heliotropin	500	(11.2 (10.	10.2
"	1000	4.7	8.4
Arbutin	500	6.8	(5.8 (5.
"	1000	8.9	(9.8 (8.9

(Table continued on page 9)

Compound.	Concentration p.p.Mil	Milligrams of Nitrogen per 100 c.c. of Ashby Media.	Control
Resorcin	500	8.12	(9.2 (8.2
Pyrogalloll	500	8.4	(8.9 (9.8
Phlorglucin	250	(5.5 (5.	8.4
"	500	7.8	10.2
Hydroquinone	500	0.0	(5.8 (5.
Salacylic Aldehyde	250	0.0	(15.8 (14.2
Oxalic Acid	500	11.2	(15.8 (14.2
" "	1000	(10.5 (11.7	(15.8 (14.2
" "	1000	8.8	10.1
" "	200	7.1	(5.8 (5.
Quinic Acid	500	10	(15.8 (14.2
" "	1000	(13 (13.7	(9.8 (8.9
" "	1000	10.7	(5.8 (5.
" "	2000	10.4	10.2
Dihydroxystearic Acid	250	(7.7 (7	(8.2 (9.2
" "	500	(7.5 (8.1	(9.8 (8.9

(Table concluded in page 10)

Table I. (Concluded).

Compound	Concentration p.p.Mil.	Milligrams of Nitrogen per 100 c.c. Ashby Media.	Control
Rhamnose	500	(8.2 (8.2	(10.2 (10.2
"	1000	(8.1 (7.6	(8.4 (8.4
Borneol	500	(8.9 (10.7	(15.8 (14.2
"	1000	(11.4 (11.2	(9.8 (8.9
"	1000	7.3	(5 (5.8
"	100	10.9	10.2

In studying compounds which contain nitrogen it was obviously necessary to take into account the amount of nitrogen added before the fixation could be measured. Accordingly, four flasks instead of two were set up with each compound in these experiments, two of the flasks receiving inoculation with Azotobacter and the remaining two used as controls for the nitrogen content of the compound. These latter flasks were kept in the incubator room during the period of incubation that they might be subjected to the same conditions as those growing the culture. It was thought that the temperature of the incubator room might have some effect on the more unstable compounds used, causing a possible loss of nitrogen. Table II summarizes the effect of some of these compounds on Azotobacter growth. The figures represent the gain in nitrogen above that added in the compound.

The Effect of Certain Nitrogenous Organic Compounds in various Concentrations on the Growth of Azotobacter.

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Compound	Concentration p. p. Mil.	Milligrams of Nitrogen Fixed per 100 c.c. Ashby's Media.	Control.
Caffeine	500	11.8	10.2
"	1000	(9.1 (9.	8.4
"	2000	(6.1 (6.5	(5.8 (5.
Betaine Hcl	500	6.8	(15.8 (14.2
Trimethyl amine	500	4.2	(9.2 (8.2
Legumin	500	8.9	(8.2 (9.2
Alloxan	500	13.6	10.2
"	1000	(6.4 (6.4	8.4
"	2000	5.02	10.1
Cinnamic Acid	500	4.5	10.2
" "	1000	7.2	8.4
" "	2000	(4.3 (4.7	(5.8 (5.
Asparaginic Acid	500	7.2	(15.8 (14.2
Asparagine	500	5.4	8.4
"	1000	5.3	8.4
"	2000	0.0	(15.8 (14.2
Hipperic Acid	500	(7.8 (8.	8.4
" "	1000	(7.7 (8.9	8.4
" "	2000	4.02	(5.8 & (5.
Creatine	500	(8.8 (7.2	(9.2 (8.2
"	1000	(5.3 (5.2	(14.4 (15.9
Creatinine	500	(8.8 (8.8	(9.2 (8.2
"	1000	7	(14.4 (15.9
Xanthine	500	5.6	(9.2 (8.2

Table II(Concluded).

Compound	Concentration p.p. Mil.	Milligrams of Nitrogen Fixed per 100 c.c. Ashby's Media.	Control.
Xanthine	1000	5.2	(14.4 (15.9
Hypoxanthine	500	4.1	(9.2 (8.2
"	1000	(2.6 (1.7	(14.4 (15.9

So strikingly did some of the nitrogenous bodies depress fixation, either through toxic properties or by affording a form of nitrogen readily utilized by the Azotobacter, that it appears convenient to list them in a separate table. They are arranged in Table III which follows:

Table III.

Nitrogenous Compounds which Strikingly Depress Fixation by Azotobacter.

Compound	Concentration p.p.Mil.	Milligrams of Nitrogen per 100 c.c. of Ashby's Media.	Control.
Urea	250	(6.6 (5.9	8.4
"	500	0.0	8.4
"	500	0.0	(14.4 (15.9
"	500	0.0	10.1
Formamide	500	(0.0 (2.2	(14.4 (15.9
"	500	2.2	(9.2 (8.2
"	500	0.0	10.1

TABLE III (Concluded)

Compound	Concentration	Milligrams of Nitrogen per 100 c.c. Ashby's Media.	Control.
Glycocoll	500	3.1	10.2
"	500	3.7	10.1
"	1000	0.0	10.2
Allantoin	500	0.0	(9.2 (8.2
"	500	(2.3 (1.5	(14.4 (15.9
"	500	2.4	10.1
Guanidine Carbonate	500	0.0	(15.8 (14.2
Nicotine	250	2.7	
"	500	0.0	(15.8 (14.2
Picoline	500	(3.1 (4.6	(9.2 (8.2
Skatol	500	3.5	(9.2 (8.2
Piperidine Hydrochloride	500	4.5	(9.2 (8.2
Sodium Nitrite	500	2.34	9.2 8.2

Parts per million, it will be noted, are milligrams per liter and concentrations of 250, 500, 1000, and 2000 p. p. Mil. represent quantities of .025, .05, .1, and .2 grams respectively. In 100 c.c. they may be regarded also as per cent. The concentration of 500 parts per million was the first used with most of the compounds as it was assumed that many of them would be decidedly toxic or fatal in this strength, and a gradation would be made downward. A consideration of Table I, however, is impressive with the indifference with which most of the compounds affect fixation. There is, to be sure, almost a uniform depression, with the notable exception of Esculin, Quinic Acid and Borneal, but it is not to the extent which might be expected from the nature of the compounds. With the exception of Pyrogalloll, Hydroquinone, Salacylic Aldehyde, and Oxalic Acid, all[a] of the compounds in Table I have been studied with reference to their effect on the growth of wheat plants[b] and all reported fatal in concentration as high as 500 p.p. Mil. and many at strengths decidedly below that figure. Their toxicity apparently does not extend to the Azotobacter. The stimulation which Esculin and Quinic Acid afford is significant. Both compounds are reported fatal to wheat seedlings at 500 parts per million, yet above

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a. The properties and reports on the toxicity of all of the compounds studied in this paper are tabulated at the end of the article.

b. Certain Organic Constituents of Soils in their Relation to Soil Fertility. Schreiner, Reed and Skinner. U.S.Dept. Agr. Bureau of Soils. Bull. No. 47.

Di-hydroxystearic Acid reported in Bureau of Soils, Bull. No. 53.

this figure they offer a striking stimulation to Azotobacter's growth. Hydroquinone and Salacylic Aldehyde present the most marked toxic effects. Yet aside from these compounds it does not appear that any of those reported in Table I are especially active in influencing fixation.

Somewhat similar to the effect noted in Table I is that evidenced by the nitrogenous bodies as tabulated in Table II - there are few instances of decided toxicity. Trimethyamine and Alloxan are most noticeable in this respect. Caffeine consistently affords stimulation. There are few of the compounds, however, from which an inhibiting effect might be expected. Indeed previous studies[a] of Asparagine, Creatine, Creatinine, Xanthine, and Hypoxanthine report them beneficial to higher plants. It is suggested that the compounds are absorbed by the plants with a beneficial effect comparable even to that afforded by nitrates. The compounds in question appear too complex to be utilized by Azotobacter as a source of nitrogen-which fact would be evidenced by no gain in nitrogen over that originally added - and neither do they exhibit any marked deleterious effect save in the higher concentrations.

In contrast to the results reported in the preceding tables are those shown in Table III. The action of the compounds here is decisive - there is little fixation in the presence of any of them and with some the process is inhibited altogether. It is clearly a condition of toxicity with the compounds Nicotine, Picoline, Skatol, ~~Guanidine~~, and Piperidine. These substances are notorious for their inimical

a. Nitrogenous Soil Constituents and their Bearing on Soil Fertility. Schreiner and Skinner. U. S. Dept. Agr. Bureau of Soils. Bul. No. 87.

effect on plant growth and their action in this case could scarcely be ascribed to any cause other than their natural toxic properties. But with such compounds as Urea, Glycocoll, Formamide, and Allantoin a possibility presents itself which may be considered as an influencing factor with many of the nitrogenous compounds, the utilization by the Azotobacter of the nitrogen supplied by the compounds in preference to that of the atmosphere.

A number of the compounds in question have been shown to be readily assimilated by the higher plants. Urea, Glycocoll, Formamide, can be utilized by peas[a] as a source of nitrogen. It is significant that these compounds so strikingly depress fixation. They present, perhaps, the simplest forms of nitrogen of any of the compounds and are therefore most readily utilized. As a result, the Azotobacter do not exercise their ability to fix atmosphere nitrogen and there is no gain noted in the final analysis. A similar explanation may be offered to account for the apparent toxicity of such compounds as Creatine Creatinine, Xanthine, Hypoxanthine, Asparagine, and Allantoin. A previous reference has noted the beneficial effect exerted by these compounds on the growth of higher plants, ascribing it to the ability of the plants to utilize the compounds as a source of nitrogen. Most of these compounds are, however, somewhat complex and it is doubtful that they are assimilated to an appreciable degree by the Azotobacter.

a. The Direct Assimilation of Inorganic and Organic Forms of Nitrogen by higher Plants. Hutchinson and Miller. Centrabl. f. Bact. Abt. II., Bd. 30. 1911. P. 513.

The interesting point of the whole condition is that the simplest nitrogenous compounds studied which are readily assimilated by higher plants and which have no general toxic properties, most uniformly depress fixation. It is suggestive of the fact that they may afford a convenient form of nitrogen for the Azotobacter and other forms of bacteria.

Summary.

The foregoing paper reports a study on the effect of various organic compounds on the growth of Azotobacter. The study was induced by the theory that the soil contains organic substances which are deleterious to plant growth and which are important factors in influencing soil fertility. It is, therefore, interesting to determine if this toxicity extends to the lower plants. The Azotobacter was chosen as a representative of the soil flora since it is of recognized importance in the maintenance of soil fertility and its growth may be accurately measured by analytical means. The compounds used were those likely to be constituents of the soil.

The results of the study indicate that fixation of nitrogen by Azotobacter is only slightly influenced by most of the compounds investigated. A depression is noted in many cases but it is usually the result of a relatively high concentration of the compound used.

Hydroquinone and Salacylic Aldehyde revealed the most toxic properties of any compounds studied.

Esculin, Quinic Acid, and Borneal afforded marked stimulation to the growth of the organism.

The effects of the compounds on Azotobacter are not, as a rule, in accord with what has been reported of their action on the higher plants. In concentrations which are fatal to certain higher plants, many of the compounds only slightly depressed fixation.

A number of nitrogenous bodies were investigated. Such compounds as Nicotine, Picoline, ~~Guanidine~~, and Skatol exhibited toxic properties commensurate to those usually ascribed to these substances.

Caffeine appeared to stimulate the growth of the organism.

Many of the nitrogenous compounds used which have been reported as beneficial to higher plants exercised a marked depression on fixation. It appears that the simpler compounds were more pronounced in this respect than were the more complex ones. It is suggested that this condition is not one of toxicity but that the nitrogen of the compounds was utilized by the Azotobacter in preference to that of the atmosphere. Urea, Glycocoll, Formamide, Guanadine, and Allantoin were especially active in depressing fixation.

Table of the Organic Compounds studied, showing their Occurrence and possible Source in the Soil, together with reports on their Action towards higher plants.

Esculin[a] $C_{15}H_{16}O_9$ has been found in the bark of chestnut and other plants. It has been reported injurious to wheat plants in concentration of 1 p. p. Mil and fatal above 500 p. p. Mil.

Vanillin[a] $C_8H_8O_3$ occurs in the vanilla bean and has been reported in oats, white lupine, raw beet sugar, and etc. It is toxic to wheat plants in practically all concentrations and fatal above 500 p. p. Mil.

Daphnetin[a] $C_9H_6O_4$ occurs in species of Daphne and is related to Cumarin. It is reported insoluble above 50 p. p. Mil. but is toxic to wheat in that concentration.

Cumarin[a] $C_9H_6O_2$ is said to occur in certain grasses, clover, beets, and other plants. It exhibits toxicity to wheat in concentration of 1 p. p. Mil. and is fatal at 100 p. p. Mil.

Pyrocatechin[a] $C_6H_4(OH)_2$ occurs in the sap of certain trees and in leaves of various plants. Concentrations of 1 p. p. Mil offered a slight stimulation to wheat; 25 p. p. Mil. caused slight injury, and 500 p. p. Mil. was fatal.

Heliotropin[a] $C_8H_6O_3$ is found in certain flowers. It is toxic to wheat in small concentrations but not fatal to growth in quantities as high as 1000 p. p. Mil.

Arbutin[a] $C_{12}H_{16}O_7$ is a glucoside of hydroquinone and is widely distributed in plants. Is reported toxic to wheat at 25 p.p. Mil. and fatal at 500 p. p. Mil.

Resorcin[a] $C_6H_4(OH)_2$ is derived from resin which is found in a number of plants.

Pyrogallol[a] $C_6H_3(OH)_3$ in solutions above 25 p. p. Mil. is reported toxic to wheat plants.

Phlorglucine[a] $C_6H_3(OH)_3$ is not found in plants but is derived from several plant constituents. It causes injury to wheat in concentrations of 25 p.p. Mil. and is fatal at 500 p. p. Mil.

Hydroquinone $C_6H_4(OH)_2$ is closely related to Quinone[a] which is sometimes found in the soil. It is toxic to wheat plants in all concentrations.

Salicylic Aldehyde[b] $C_7H_6O_2$ occurs in the blossoms of certain plants. It has also been isolated from the soil.

Oxalic Acid $C_2H_2O_4$ has been found in soils probably as Calcium Oxalate. It is a common product of decomposing organic plant constituents.

Quinic Acid[a] $C_6H_7(OH)_4$ COOH is found in cinchona bark always accompanying Quinine. It is reported to stimulate wheat below concentrations of 500 p.p. Mil. but is extremely toxic above 500 p.p. Mil.

Di-hydroxystearic Acid[c] has been isolated from a number of soils. It is reported toxic to wheat plants in very minute concentrations.

Rhamnose[b] $C_6H_{12}O_5$ has been derived from a glucoside isolated from the soil. It may also be obtained from a number of glucosides which occur widespread in plants.

Borneol[a] $C_{10}H_{12}OH$ is a representative of the camphor group and occurs in needles of pine and fir trees. It is quite insoluble. It is reported toxic to wheat at 1 p.p. Mil. and fatal at 100 p.p. Mil.

Caffeine $C_8H_{10}O_2N_4$ is found in leaves and beans of the coffee tree, in tea, cocoa, and etc. It is closely related to Xanthine.

Betaine[a] $C_3H_5O_2N$ occurs in the juice of sugar beets and in many seeds and plants. It is reported beneficial to wheat in concentrations from 5 to 1000 p. p. Mil.

Trimethylamine[b] C_3H_9N. has been isolated from soils. It occurs in plant and animal tissues.

Legumin occurs in the seeds of a number of plants especially the lupines.

Alloxan[a] $CO\left\langle\begin{matrix}NH\ CO\\NH\ CO\end{matrix}\right\rangle CO$. is closely related to compounds which occur in plants. It is readily assimilated by peas[d]. It is reported toxic to wheat above 100 p. p. Mil.

Cinnamic Acid[a] C_6H_5 CH:CH.COOH. is found in resin balsam, storax, and occurs in the decomposition of certain alkaloids. It is reported toxic to wheat at 25 p. p. Mil. and fatal above 100 p.p.Mil.

Asparaginic Acid[a] $COOH.CH_2\ CH(NH)_2\ COOH$. is found in young sugar cane and beets and in the seed of various plants. It is fatal to wheat plants in concentrations of 500 p.p.Mil.

Asparagine[a] $NH_2OC.\ CH_2\ CH(NH_2)\ COOH$. occurs in young shoots of Asparagus plants and in many other plants. It is reported favorable to wheat grown in solutions without nitrate. Its beneficial action decreases with increased nitrates.

Hippuric Acid[d] $CH_2\ NH.CO.C_6H_5.COOH$. Occurs in the urine of herbivorous animals. It is reported only slightly assimilated by peas.

Creatine ($C_4H_9N_3O_2$) and Creatinine[e] ($C_4H_7N_3O$) are closely related ~~$C_4H_9N_3O_2$ $C_4H_7N_3O$~~. Chemically. The latter occurs in soils, is widely distributed in seeds and is a constituent of manures and animal flesh. Both are oxidation products of Guanidine. They have been shown to exert a beneficial effect on wheat plants[f]. The stimulation is not so marked in the presence of nitrates.

Xanthine[g] $C_5H_4N_4O_2$ and Hypoxathine[f] ($C_5H_4N_4O$) are closely related chemically and occur widely in soils. They are related to Uric Acid. They have been reported favorable to the growth of wheat.

Urea[d] $CO\begin{smallmatrix}NH_2\\NH_2\end{smallmatrix}$ occurs in the excreta of animals and therefore in manures. It is reported as readily assimilated by peas.

Formamide[d] $CO\begin{smallmatrix}H\\NH_2\end{smallmatrix}$ is reported assimilated by peas.

References from the foregoing Table

a. Certain Organic Constituents of Soils in Relation to Soil Fertility. Schreiner, Reed and Skinner. U. S. Dept. Agr., Bureau of Soils. Bulletin No. 47.

b. Some Organic Soil Constituents. Edmund C. Shorey. U. S. Dept. Agr. Bureau of Soils. Bulletin No. 88.

c. Examination of Soils for Organic Constituents, especially Dihydroxystearic Acid. Schreiner and Lathrop. U. S. Dept. Agr. Bureau of Soils. Bulletin No. 80.

d. The Direct Assimilation of Inorganic and Organic Forms of Nitrogen by Higher Plants. Hutchinson and Miller. Centrbl. f. Bact. Abt II. Bd. 30. P.513.

e. A Beneficial Organic Constituent of Soils: Creatinine. U. S. Dept. Agr. Bureau of Soils. Bulletin No. 83.

f. Nitrogenous Soil Constituents and their Bearing on Soil Fertility. Schreiner and Skinner. U. S. Dept. Agr. Bureau of Soils. Bulletin No. 87.