DECAY AND SOIL TOXINS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 213

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The decomposition products of a specific plant organ and their effects on the growth of other plants furnished the point of attack for the work on toxicity reported in this paper. The material used was the rhizomes of *Nymphaea advena* Ait. and *N. polysepala* Greene. This material was obtained at intervals from July 1912 to October 1915, at various places in the vicinity of Chicago, Illinois, and Seattle, Washington.

Review of literature

RELATED WORK ON TOXICITY

The organic constituents of soils have been under investigation by workers in the United States Bureau of Soils for 10 years. LIVINGSTON (12, 13) found toxic substances, probably organic, in an unproductive soil. SCHREINER (21) and his co-workers have isolated from soils more than 25 organic compounds differing widely in chemical character. Some of these (for example, dihydroxystearic acid) have proved harmful to growing plants; some (for example, nucleic acid) have been found beneficial; and some have not been shown to have any effect on the growth of plants. BOTTOMLY (3) has found that certain aerobic organisms grow well in peat and form from it compounds that are beneficial to the growth of plants. He suggests that very small amounts of accessory organic substances may be necessary for the growth of plants.

Humic acid has been much discussed as a possible factor in plant growth. Not only the effects of this so-called humic acid, but also the constitution and nature of the substance are in doubt. SCHREINER (21) regards it as a mixture of substances. WIELER (29) takes the view that humic acids in soils are inorganic acids resulting, for example, from the chemical decomposition of salts.

BAUMAN and GULLY (2) have suggested that the acidity of bog water is due to the fact that the cell colloids of the disintegrating

plant tissues retain chiefly the basic ions of the salts dissolved in the cell contents of the plant tissues before they began to decay, thus freeing the acid ions. SKENE (24) has found that various species of *Sphagnum* thrive best in acid solution because mineral solutions, although usually physiologically harmless, may be ecologically harmful.

Work by LIVINGSTON (14), DACHNOWSKI (6, 7), the writer (19), and others (20), indicates that the inhibition from sphagnum bogs of plants other than bog xerophytes is not due to acidity, or to low surface tension, or to high osmotic pressure of the soil solution, but is due in part to the presence of toxic substance or substances in the soil solution.

Many workers (8, 9, 15–18, 25–27) have found that cultivated crops and plants grown in cultures have a favorable or an unfavorable influence on other plants growing in the same substratum either at the same time or subsequently. Food supply and toxins have been suggested as means through which this influence may be exerted. CZAPEK (5) finds that the roots of plants are injured when the surface tension of the bathing solution is lower than 0.66.

SHERFF (23) found in Skokie marsh near Chicago that where the rhizomes of *Sagittaria latifolia* had penetrated the decaying rhizomes of *Nymphaea advena*, they themselves had begun to decay.

STERILE CULTURES OF SEED PLANTS

More or less success has been attained by various workers in attempts to grow seed plants under sterile conditions. HARRISON and BARLOW (11) tried sterilization by dry heat, moist heat, sulphuric acid, calcium hydrate, formaldehyde, and mercuric chloride, and abandoned all of these means. They succeeded in getting sterile cultures of certain legumes by treating the unopened pods with mercuric chloride, opening them with flamed forceps, and transferring the seeds to a very small quantity of boiling water in sterile test tubes.

WILSON and HARDING (30) tried alcohol, formaldehyde, and mercuric chloride as a means of sterilizing alfalfa seeds, but found that when the seeds were sterile, the germination was very low.

Using a modification of HARRISON and BARLOW'S method, they got alfalfa seedlings which grew in sterile cultures for 4 months.

BROWN (4) found that barley seeds take up water from a fairly strong solution of sulphuric acid and remain uninjured. SCHROEDER (22) found silver nitrate to be a good means of sterilizing wheat. He found that hand-picked wheat endured soaking in a 5 per cent solution of this substance for 24 hours without injury, and for 72 hours with but slight injury. Threshed wheat, however, because of the rupture of semipermeable membranes by the machinery, would not stand such prolonged treatment. ARCHICHOWSKY (1) got a large percentage of sterile cultures of seed plants by the use of formaldehyde and other antiseptic agents on peas, pumpkins, and other seeds.

Solutions and preparations

Both of the species of *Nymphaea* used produce branched rhizomes 3–15 cm. thick and sometimes reaching as great a length as 3 m., although they are more commonly 1 m. or less. The older portions of these rhizomes decay. SHERFF (23) found these rhizomes decaying to within a short distance of the growing apex. The writer has found the decay only in older portions of the rhizome. Sound pieces of the rhizome were collected and the following solutions were made up quantitatively, each solution having a volume of 1600 cc. and containing the solutes obtained by the methods described from 1000 gm. of fresh rhizome. An average of 3 tests on the water contents of the fresh rhizome gives 88 per cent of water. The tests were made by cutting 500 gm. of the fresh material into small pieces and drying it at 105° C.

Solution 1A.—This was the liquid resulting from the decay of 1000 gm. of fresh *Nymphaea* rhizome in redistilled water, freed from solid matter by filtering through cheesecloth, and diluted to 1600 cc. with redistilled water. Molds continued to grow on the surface of this solution. It was amber colored.

Solution 1B.—This was the solution remaining after a duplicate of 1A had been extracted by shaking with an equal volume of ether in a separatory funnel. The ether that dissolved in the water solution was removed by heating to 40° C. and subjecting to

a suction of 2 cm. of mercury with an aspirator. Molds continued to grow on the surface of this solution. It had a slightly darker color than 1A.

Solution 1C.—The ether used in extracting 1B was allowed to evaporate spontaneously. The solid remaining was reddish-brown, and only partially soluble in water. This solution consists of 1600 cc. of redistilled water, and all the residue from the ether extraction that would dissolve in that quantity of water at 40° C. No molds grew on this solution, and no scum or turbidity or other evidence of bacterial activity appeared.

Preparation 1D.—This was the solid remaining from filtering 1A. This solid was ground with an equal volume of sand.

Solution 2A.—This was the distillate under reduced pressure (2 cm. pressure), at 40° C., of the liquid and solid products of the decay of 1000 gm. of *Nymphaea* rhizome in redistilled water. This was a clear liquid having the appearance of water. No molds grew upon it, and it showed no evidence of bacterial activity.

Solution 2B.—The solid remaining from the distillation of 2A was dried in an oven at 30° C. and then ground in a mortar. It was then black powder. This was extracted in a Soxhlet apparatus with ether. When the ether was allowed to evaporate spontaneously, a sticky, semi-solid, reddish-yellow substance remained. This was only partially soluble in water. This solution represents 1600 cc. of redistilled water, with all of the ether extract that would dissolve in it at 40° C. It had a light reddish-yellow color. No molds grew upon it, and no evidence of bacterial activity appeared.

Solution 2C.—The solid remaining from the extract of 2B was exposed to air until all odor of ether had disappeared. It was then extracted for 2 hours with 1600 cc. of redistilled water. It was perfectly clear. No molds or evidence of bacterial growth appeared.

Preparation 2D.—This represents the solid remaining from the extract of 2C, ground in a mortar with an equal volume of sand to form a soil.

Solution 3A.—One kg. of fresh *Nymphaea* rhizome was cut into pieces, ground in a meat grinder, and the juice pressed out in a fruit press. The solid remaining was extracted with ether, and

afterward exposed to the air until the odor of ether disappeared. It was then allowed to decay in redistilled water. The liquid resulting from this decay was strained through cheesecloth and diluted with redistilled water to 1600 cc.

Solution 3B.—The juice squeezed out in preparing solution 4A was extracted by shaking with an equal volume of ether in a separatory funnel, which was then freed from ether by heating to 40° C., and subjecting to suction (2 cm. of mercury) by means of an aspirator for 24 hours. This, when diluted 1600 cc., constituted solution 3B.

Solution 3C.—The ether used in extracting 3A was combined with that used in extracting 3B. This was allowed to evaporate spontaneously and as much of the residue as possible was taken up in 1600 cc. of redistilled water at 40° C.

Preparation 3D.—The solid matter remaining on the cheesecloth in the preparation of 3A was air-dried and ground with an equal volume of sand to form a soil.

Solution 4A.—This was the water extract of the fresh rhizome made under sterile conditions. The rhizome was cut into small pieces and placed in flasks with water. These flasks were stoppered with cotton and sterilized in an autoclave. This solution stood sterile for 11 months before its toxicity was tested.

Solution 5A.—This was the water solution of the ash from the fresh rhizome.

All sand used in the previous preparations and in the following experiments was either no. $2\frac{1}{2}$ quartz or "Ottawa test." In all cases it was washed in 10 per cent HCl, freed from acid by washing in running water, and finally rinsed with redistilled water.

The "Knop's solution" had the following composition: 1 part KNO_3; 1 part K_2HPO_4; 1 part $Mg\,SO_4$; 4 parts $Ca(NO_3)_2$. This was made up to 0.1 per cent.

Where "tap water" is mentioned, the water used was Chicago city water. Where "Cedar River water" is mentioned, the water used was Seattle city water, which is piped from the river near its origin in a snow-fed lake. Where "Lake Washington water" is mentioned, the water is that supplied from Lake Washington to the botany laboratories at the University of Washington.

Experiments and results

In order to determine the relative toxicity of these various solutions to *Tradescantia*, cuttings of the plant were placed in various dilutions of each solution with redistilled water. In this way the percentage of the solution (that is, the number of cc. diluted to 100 with redistilled water) that would allow the formation of roots but inhibit the production of root hairs was determined.

All of the solutions except 5A were acid to both litmus and phenolphthalein. Their acidity was determined by titrating with N/10 NaOH, using phenolphthalein as an indicator.

Table I gives the toxic limits of these solutions to *Tradescantia* (as previously defined) and their acidity, together with the relative rank of each solution as to toxicity and acidity and the ratios of these.

TABLE I

Solution acidity	Toxicity (per cent)	Rank as to acidity	Rank as to toxicity	Ratio of acidity to toxicity
1A N25/1000	7.5	1	1, 2, or 3	0.27
1B N24/1000	7.5	2	1, 2, or 3	0.25
1C N11/1000	7.5	4	1, 2, or 3	0.12
2A N22/1000	10.0	3	4	0.24
2B N6 /1000	50.0	7	7	0.12
3A N9 /1000	12.5	5	5	0.10
3B N7 /1000	15.0	6	6	0.08
3C N5 /1000	75.0	8	8	0.06

All of the solutions mentioned in table I, except 3B, were neutralized to phenolphthalein with N/10 sodium hydrate, and the effect of both the acid and the neutral solution was tried on *Tradescantia* cuttings. Table II shows the results of the dilutions named on *Tradescantia* cuttings.

TABLE II

Solution	Strength (per cent)	Acid	Neutralized
1A	7.5	Root hairs none	Root hairs normal
1B	5.0	" " slightly stunted	" " "
1C	5.0	" " " "	" " normal
2A	7.5	" " none	" " slightly stunted
2B	60.0	Roots none	Roots 3–10 mm. long
3A	12.5	Root hairs none	Root hairs slightly stunted
3C	10.0	" " slightly stunted	" " normal

The toxicity of the first 4 of these solutions when undiluted was not perceptibly lowered by neutralization with sodium hydrate.

A 20 per cent solution of 1A was shaken in a large flask 5 times a day for 10 minutes each time, for 3 days in order to aerate it, and then filtered through a filter paper. Tests on *Tradescantia* indicated that its toxicity was not decreased by this treatment.

A 20 per cent solution of solution A in tap water was shaken with animal charcoal and filtered. The filtrate was colorless and odorless. The solution before this treatment had an amber color and a foul odor. Cuttings of *Tradescantia* were placed in this. In all cases they grew well and produced good roots with normal or only slightly stunted root hairs.

The animal charcoal with which this solution was shaken was used as a soil for cultures of alfalfa seed. Controls of animal charcoal shaken with tap water were run. The growth in the controls was twice as great as the growth in the cultures.

The toxicity of preparation 2D to *Tradescantia* cuttings was tested by planting the cuttings in the preparation in small flower pots. This preparation was further diluted also by grinding with half its volume of sand. For convenience this further dilution is referred to in table III as preparation 2Dx. Controls were run

TABLE III

Medium	Roots	Plants
2D	None	Dying
2Dx	Normal	Healthy
Potting soil	Normal	Healthy

in potting soil. All of the pots in a set were placed together in a large glass dish, so that they stood in about one-fourth of an inch of tap water and thus were all watered alike. Table III shows the results at the end of 14 days.

Preparation 3D was tried in the same way with similar results. Preparation 1D was tried in the same way with corn and did not prove toxic. Preparations 2D and 3D are not at all toxic to alfalfa.

The toxicity of solutions 1A, 1B, 1C, and 2A to unsterilized corn was tested by planting the seeds in 200 cc. of sand watered with 25 cc. of solution. The cultures were in 500 cc. Erlenmeyer

flasks, stoppered with cotton. Controls watered with Knop's solution were run. All of the solutions tested proved toxic, but 2A was found to be less toxic to corn under these conditions than the others. The toxicity of the solutions was shown in the injury to the root tips, causing a great decrease in the total length of root produced as compared with the controls, and thus eventually killing the plants.

The effect of the 8 solutions listed in table I was tried on unsterilized alfalfa in sand. They were all found extremely toxic, many of the plants dying as they emerged from the sand, and the best of them attaining only one-fifth of the height attained by controls watered with tap water.

It was found by tests that tomato seeds did not germinate at all in solution 1A in sand, while controls in sand watered with tap water grew vigorously.

An attempt was made to secure sterile cultures of corn in 500 cc. Erlenmeyer flasks in order to determine whether the presence of organisms in the cultures was a factor in the toxicity of these cultures. The sterility of the cultures was tested by making bouillon cultures from various portions of the sand and the seeds at the end of the experiment; 65 per cent of the cultures proved sterile.

Three problems were to be solved in working out a method of securing sterile cultures of seed plants: (1) the sterilization of the flasks and contents; (2) the sterilization of the seeds; (3) the transfer of the sterile seeds to the sterile flasks under sterile conditions.

The cotton-stoppered flasks, containing 200 cc. of sand and 20 cc. of the solution, were sterilized for 1 hour at 15 lbs. pressure in the autoclave. A solution of silver nitrate was used as a means of sterilizing the seeds. It was found by experiment that corn would germinate well after treatment for 1 minute with N/300 $AgNO_3$ and subsequent washing with water. As a means of making the transfer of the seeds to the flask, the box previously used by JENSEN (**11a**) was used. When the lid of the box was closed, the operator could thrust his hands into the gloves and work without danger of contaminating the cultures from any source outside of the box. Since the entire top and part of the front of

the box were of glass, all articles inside of the box could be seen readily by the operator.

The entire inside of the box, including the side of the gloves and sleeves exposed to the air of the box, and the outside of all flasks and other articles placed in the box, were treated thoroughly with a 15 per cent solution of glycerine saturated with carbolic acid. All of the cultures prepared were thus more or less exposed to carbolic acid fumes. This solution was applied by means of a sponge attached to a short stick. It was found that flasks and dishes stuck to the paint treated with this mixture, so that a false bottom of glass was placed in the box. The stoppers of the sterilized flasks (including 2 of sterile water) were flamed, and the flasks placed in the box. Four tall stenders with ground glass covers were sterilized in an oven for 5 hours at 120° C., and placed in the box. A bottle of N/300 $AgNo_3$ was also placed in the box, as were also a pair of 10-inch brass forceps and a waste jar containing a little of the glycerine carbolic acid solution. Everything in the box having been treated with the antiseptic solution, the cover of one of the stenders was removed and dry corn placed in the stender and the cover quickly replaced. In selecting corn for this purpose, care had been taken to secure smooth kernels that would offer little opportunity for the lodgment of air bubbles in the dent or elsewhere.

The box now remained closed over night, to allow any organisms in the air to settle to the bottom of the box and be held by the glycerine solution. In the morning the operator thrust his hands into the gloves, poured $AgNO_3$ on the seeds, left it on for 1 minute, poured it off into the waste jar, and then washed the seeds in several changes of sterile water, finally allowing them to soak in it for several hours, and rinsing them again. The tips of the forceps now were washed thoroughly in the sterile water and the transfer of the seeds was made. In some cultures the seeds were thrust down into the sand by means of these forceps, and in some cases they were left on the surface. After 2 or 3 cultures had been made, the forceps were placed in the antiseptic solution for a few minutes and again rinsed in sterile water. After the cultures were prepared, they were removed from the box and placed near a window in the laboratory.

The results showed that solutions 1A, 1B, 1C, and 2A (these were the only ones tried) are just as toxic under these conditions as when organisms were known to be present in abundance. Since 65 per cent of these cultures were shown (by bouillon cultures from them at the close of the experiment) to be sterile, it is evident that the presence of organisms is not a necessary condition of the toxicity of these solutions.

Solutions 1A, 1B, and 1C likewise proved toxic to alfalfa in cultures prepared as previously described for corn. The same precautions for securing sterility were observed, but tests of sterility were not made.

The toxicity of solution 4A to *Tradescantia* cuttings was tested as follows: wide-mouth 50 cc. bottles were filled with dilutions of the following strength: 20, 15, and 10 per cent. Into each of these was placed a cutting of *Tradescantia*. The mouths of the bottles were left open. At the end of 16 days no root hairs had formed on any of these plants and all of the plants were showing signs of death. The controls in Lake Washington water all had abundant root hairs and were in healthy condition.

Other sets of bottles were prepared as previously described, except that the mouths were stoppered with cotton and the bottles containing the liquids were sterilized in the autoclave. The dilutions were as follows: undiluted, 20, 15, and 10 per cent. Each cotton stopper was then displaced just enough to allow a cutting of *Tradescantia* to be placed in the liquid. None of these plants developed normal root hairs. All of them except those in the 10 per cent dilution showed signs of death at the end of 16 days. Turbidity due to the action of organisms was evident in all of these, and molds grew on some of them.

Flasks (500 cc.) were prepared, cotton-stoppered, each containing 180 gm. of sand and 18 cc. of solution. These were autoclaved at 12 lbs. pressure for 1 hour. Solution 4A was used pure and also in the following solutions: 20, 15, and 10 per cent. Controls with tap water were also run. Corn was treated with N/100 silver nitrate for 2 minutes, then with sterile forceps 5 kernels were placed in each flask. At the end of 16 days the growth was noticeably greater in all of the controls than in any of the solutions. All

of the flasks except 2 (one control and one 15 per cent) had molds growing in them. Bouillon and also agar cultures were made from each of these, and the 15 per cent flask proved to be sterile. The other was not sterile. These 2 flasks were kept a week longer and the growth in the control was much better than that in the 15 per cent solution.

The transfers of the corn in these cases were made in the laboratory, without the use of the sterile box just described. It is hoped that by autoclaving the flasks at a higher pressure, and treating the seeds with the silver nitrate for a longer period, a larger number of sterile flasks could be obtained.

Solution 1A was filtered and the fresh filtrate was immediately saturated with ammonium sulphate. The sulphate was added gradually and the solution was shaken after each addition. No precipitate appeared at once, but when it had stood over night a considerable amount of a brownish precipitate was present. Some of the precipitate was at the surface of the liquid, some had settled to the bottom, and some particles were in suspension in the liquid.

The precipitate was filtered off and redissolved in a volume of Cedar River water equal to the original volume of the solution. Both filtrate and precipitate were then dialyzed in dialyzing tubing in running water for 11 days. At the end of this time they showed no precipitate with barium chloride. The filtrate and the precipitate were then tested for toxicity by placing *Tradescantia* cuttings in them. Root hairs were formed in both, but their development was poorer in the solution of the precipitate than in the filtrate.

In preparing solution 5A, 2.83 gm. of ash were obtained from 500 gm. of fresh rhizomes. This is 4.7 per cent of the dry weight. Approximately half of this went into solution when shaken with 800 cc. of Cedar River water at 18° C. The solution was basic to litmus. In all cases tried with 5A and its duplicates the toxicity to *Tradescantia* cuttings was so marked that practically no root hairs developed and the plants soon died. When dilutions were tried it was found that all dilutions down to 10 per cent (10 cc. solution to 90 cc. water) inhibited root hair production; in 5 per cent dilution root hairs developed normally.

A solution was prepared from each of the following substances, by allowing a quantity of it to decay in redistilled water: potato, turnip, rhizome of *Castilia odorata*, and rhizome of *Typha latifolia*. These solutions approximated the strength of solution 1A prepared from *Nymphaea*. They all proved toxic to *Tradescantia* cuttings, but to a less degree than 1A did. Their toxicity was in the order named.

Discussion

It is evident from the data given that even very dilute solutions of the products of the decay of *Nymphaea* rhizomes are toxic to *Tradescantia* cuttings in water cultures and the seedlings of tomato, alfalfa, and corn in sand cultures.

Although the products of the decay of the subterranean parts of other plants proved toxic, the toxicity of the products of the decay of *Nymphaea* rhizomes was considerably greater than that of any other plant parts experimented on. While it is possible that toxicity from decay is rather common, *Nymphaea* seems to merit particular attention in this regard. The dilution of solution 1A that entirely inhibited the formation of root hairs on *Tradescantia* cuttings contained in each cc. the products of the decay of 4.7 mg. of fresh rhizome. Since only 12 per cent of the fresh rhizome is solid matter, the amount of solid whose decay contributed to the solutions in each cc. of the toxic solution was 0.56 mg.

The fact that the solutions listed in table I were all acid, and that their toxicity was largely destroyed by neutralization with sodium hydrate, would seem to suggest acidity as a large factor in the toxicity. The toxicity is not proportional to the acidity as determined by the titration method. It may be proportional, however, to the H ion concentration, or some other factor may be effective. The fact that the toxicity of 1A, 1B, 1C, and 2A when undiluted was not reduced by neutralization with sodium hydrate seems to emphasize further the presence of some other factor. It is possible that the osmotic pressure of such a concentrated solution was high enough to cause injury, although it has been shown elsewhere (**20**) that this is not the cause of the toxicity of the very dilute solutions. Antagonistic action on permeability might also

be a possible factor in the lowering of toxicity on the addition of sodium hydrate.

The fact that the toxicity of solution 1A was not destroyed by the aeration here reported does not necessarily mean that the toxicity cannot be removed by oxidation.

The removal of the toxicity from solution 1A by shaking it with animal charcoal is probably to be explained as an absorption phenomenon.

The only importance attaching to the toxicity of the soils (preparations 1D, 2D, and 3D) is that not all of the water-soluble toxic materials had been washed out of them by the treatments to which they had been subjected.

The products of the decay of *Nymphaea* rhizomes are toxic, not only to *Sagittaria* and *Tradescantia*, but also to agricultural plants. Apparently the toxicity of solution 1A to tomato, alfalfa, and corn is in the order named.

While the box used for transferring the sterile seeds to sterile flask cultures is fairly efficient, the method is somewhat slow and tedious, and it is believed that fairly good results may be obtained without its use. It seems very desirable to extend our knowledge of the growth of seed plants in the absence of other organisms.

In the one case mentioned, the toxicity of the products of this rhizome to corn seemed to be independent of the presence of organisms, either at the time of dissolving the toxin from the rhizome, or at the time of its action on the growing plants.

The fact that the toxicity of solution 1A, even when undiluted, can be removed by precipitation with ammonium sulphate seems to suggest that the presence of colloidal matter may be a considerable factor in toxicity of that solution.

An alkaloid similar to nupharin is reported by WEHMER (28) in the rhizome of *N. alba*. He also reports fat and organic acids. These also represent possible factors in the toxicity. It seems possible that the toxicity may be due partly to products formed during decay and partly to products merely released by this decay.

The toxicity of the water extract of the ash is possibly accounted for on the basis of the presence of one or more basic substances. WEHMER states that the rhizome of *Nymphaea alba* contains

9.86 per cent of ash with the following composition: CaO, 8.2 per cent; Cl, 15.2 per cent; Na_2O, 48.47 per cent; K_2O, 9.86 per cent; P_2O_5, 14.36 per cent. The presence of sodium hydroxide or of sodium carbonate in the water solution of this ash seems probable.

There seemed to be 5 possibilities as to the cause of the toxicity of the products of the decay of this rhizome: (1) the presence of the organisms; (2) toxicity due to ionization; (3) the presence of toxic molecules; (4) high osmotic pressure of the solutions; and (5) low surface tension of the solutions. Of these, (1) seems to be practically eliminated by the work here reported. Apparently the presence of organisms, either at the time of the formation of the solution or at the time of their action on growing plants, is not a condition necessary for their toxic action. Work elsewhere reported (**20**) disposes of (4) and (5). This leaves ionization and toxic molecules as probable causes of the toxicity. The relative importance of these two is not fully determined by the work here reported. It is evident, however, that the entire toxicity cannot be ascribed to one substance. If we should suppose that the toxicity of all of the solutions obtained from this rhizome was due to only one substance, it would have to have the following properties: (1) soluble in water; (2) soluble in ether; (3) volatile at 40° C., 20 mm. pressure; (4) stable at 250° C., 15 lbs. pressure; (5) absorbed by animal charcoal; and (6) precipitation by ammonium sulphate.

It seems probable that there are at least 3 classes of substances here that are somewhat toxic: (1) colloids, (2) very volatile substances, and (3) certain bases.

Summary

1. The products of the decay of *Nymphaea* rhizomes are toxic to *Tradescantia* cuttings, and to tomato, alfalfa, and corn, even in very dilute solutions.

2. All of the solutions prepared except the one from the ash were acid, but the amount of acidity was not proportional to the degree of toxicity.

3. The toxicity of the very dilute solutions (but not of the strong solutions) was nearly destroyed by neutralization with sodium hydrate.

4. The toxicity of solutions resulting directly from the decay of the rhizome is destroyed by shaking them with animal charcoal.

5. The water extract of the fresh rhizome made under 15 lb. pressure in an autoclave is toxic to growing plants.

6. The rhizome and the products of its decay contain some ether-soluble toxic material.

7. Some of the toxic material in the products of the decay of these rhizomes is volatile at 40° C.

8. The toxicity of even concentrated solutions resulting directly from the decay of these rhizomes can be removed by precipitation with ammonium sulphate.

9. The water extract of the ash from these rhizomes is basic and toxic.

10. The products of the decay of potatoes, of turnips, and of the rhizomes of *Castalia odorata* and *Typha latifolia* are also toxic to *Tradescantia*, but slightly less so than those of *Nymphaea*.

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