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STUDIES OF THE AMMONIA-SOLUBLE ORGANIC MATTER OF THE SOIL

BY

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STUDIES OF THE AMMONIA-SOLUBLE ORGANIC MATTER OF THE SOIL.

G. S. FRAPS, Chemist.
N. C. HAMNER, Assistant Chemist.

Part I—Estimation of Humus.

Part II—Formation of Humus.

Part III—Composition and Properties of the Humus.

Under the Adams Act of the United States government, providing for scientific investigations by the Experiment Stations, the Chemical Division of the Texas Station has undertaken extensive soil studies. A portion of these studies is concerned with the organic matter of the soil, and this bulletin presents some of the results of the investigation. This bulletin is strictly technical, and intended for scientific readers only. The ultimate objects are for practical agriculture, but the means and methods of the investigation must be discussed in technical language.

The importance of estimating the organic matter of the soil has been recognized for a long time. Methods based on the complete oxidation of the carbon were used, and are still in use. The total carbonaceous matter is calculated on the assumption that it contains 58 per cent of carbon, though the precentage of carbon in the organic matter of the soil is known to vary widely.

All the organic matter of the soil is not in the same condition, but exists in various stages of decomposition. This fact has also been recognized for a long time. It has been held that the black or dark-brown organic material in the soil, resulting from the decay of animal or vegetable matter introduced in the soil, has a much higher agricultural value than the undecomposed organic matter. We have not been able to discover experimental evidence in support of such opinion. It is well known that the organic materials in the soil are mixtures of various compounds, and are constantly undergoing change. Material is present in all stages of decomposition, from the original unchanged compounds found in plants or animals, to the product of complete oxidation (carbon dioxide), which is no longer organic in nature.

The organic matter soluble in ammonia is supposed to represent the decomposed organic matter of the soil, and, therefore, if the theory cited above is correct, the more valuable organic matter. The relation between the two forms of organic matter is largely a matter of color; while the ammonia does not extract all the organic matter from the soil, it does extract practically all the black organic matter, leaving the soil residue nearly white.

The organic matter of the soil soluble in ammonia is termed *humus* in America. The fact that the ammonia-soluble organic matter is of more value than that not soluble in ammonia, has not, in our opinion, been established. In Germany, the ammonia-soluble organic matter is not estimated, but the total organic matter is judged from the total amount of carbon in the soil.

The term *humus* is used in this paper for convenience in designating the ammonia-soluble organic matter, and in using this term in this way we do not mean to subscribe to any theory concerning the importance of the ammonia-soluble material.

PART I—ESTIMATION OF HUMUS

The estimation of ammonia-soluble organic matter is based upon the work of Grandeau. (Compt. rend., 1872, 988.) He said that the black earth of Russia, which is a very productive and durable soil, contained 0.20 per cent phosphoric acid, and 0.16 per cent was soluble in ammonia with the organic matter. Grandeau decided that the fertility of the soil was closely related to its content of mineral matter extracted with the organic matter soluble in ammonia. He considered the phosphoric acid combined with humus one of the most important parts of the soil.

The theories of Grandeau have found little acceptance abroad, though they have been accepted by a number of chemists in this country.

Snyder (Bulletin 30, Minnesota Experiment Station) compares three cultivated soils with three virgin soils from the same locality, and finds that the latter contain more humus, more nitrogen, and more phosphoric acid associated with the humus.

	Humus Per Cent.	Humus Phosphoric Acid Per Cent.
Warren—Native	5.34	.07
Cultivated	3.02	.03
Crookston—Native	5.16	.06
Cultivated	2.87	.03
Marshall—Native	5.12	.05
Cultivated	2.60	.03

In Bulletin 40 of the Minnesota Experiment Station, Snyder compares two more virgin soils with two cultivated soils, finding here also that the phosphoric acid decreased as the humus decreased.

	Humus Per Cent.	Humus Phosphoric Acid Per Cent.
Wilkins County, soil cultivated 2 years	5.30	.05
Cultivated 10 years	3.38	.03
Chippewa County, native soil	3.97	.07
Cultivated 23 years	2.59	.03
Dakota County, soil cultivated 35 years	2.45	.03
Cultivated 42 years	3.46	.03

He makes the following remark in regard to the mineral matter associated with humus: "The humus materials, usually known as humic acid, when extracted with a 2.5 per cent solution of ammonium hydrate or any other dilute alkali, and then precipitated with acid, yield from 5 to 25 per cent, according to the nature of the soil, of a brownish red ash. This ash is evidently in chemical combination, because if merely soluble in the alkaline solutions used for extraction, the mineral matter would not be precipitated with hydrochloric acid, but would be removed in the filtrate and washing solutions employed." The average composition of eight samples of ash from good productive soils yielding 2.5 per cent humus was found by Snyder to be as follows:

	Per Cent.
Silica	61.97
Potash	7.50
Soda	8.13
Lime	.09
Magnesia	.36
Ferric oxide	3.12
Alumina	3.48
Phosphoric acid	12.37
Sulphuric acid	.98
Carbonic acid	1.64

In Bulletin 53 of the Minnesota Experiment Station, Snyder reports a study of the effect of decaying organic matter upon the mineral matter of the soil, in which it appears that the amount of phosphoric acid and potash soluble along with the humus is increased by the action of decaying organic matter in a period extending over a year. "Cow manure, green clover, and meat scraps produce valuable forms of humus, rich in nitrogen. The humus produced is capable of combining with the phosphoric acid and potash of the soil to form humates." Bulletin 89 (1905) contains similar studies with glutin, gliadin, etc., in which the same conclusions are reached.

Ladd (Bulletin 35, North Dakota Station) found in a new soil 0.192 per cent phosphoric acid soluble with humus, and in an old soil similar to the soil first named 0.179 per cent. The humus in the new soil was 2.53 per cent, in the old soil 1.56 per cent. Ladd concludes that the cropping decreases the humic phosphoric acid and the humus. The difference in phosphoric acid soluble with humus in these soils, however, is no greater than might naturally occur, due to error of analysis, error of sampling, and difference in the original soils.

The humus and humus phosphoric acid were determined by Ladd at different periods, in a crop rotation plot, with the following results:

	Humus Per Cent.	Phosphoric with Humus Per Cent.
1891	5.35	.079
1894	6.82	.091
1896	7.86	.117

Ladd also made analyses of the humus extract from twenty-four soils. A summary of the analyses is as follows:

	Percentage in soil		
	Average	Maximum	Minimum
Humates	9.150	15.260	3.84
Humus	4.770	7.900	1.56
Total Phosphoric Acid	.269	.400	Trace
Phosphoric Acid in Humus	.138	.199	Trace
Percentage of total Phosphoric Acid in Humus	51.300	112 (?)	Trace
Percentage of organic matter in Humates	52.100	70.800	40.60

By "humates" Ladd means the total amount of material removed with the ammonia.

Snyder (Bulletin 89, Minnesota Experiment Station) claims that gliadin and egg albumen, in decaying in a soil, increase the phosphoric acid soluble with humus.

METHODS OF ANALYSIS.

In the estimation of humus, the humates of the soil are decomposed with acids, thereby setting free the humic acids and extracting the lime. The soil is then treated with ammonia, the clay allowed to settle, and the humate solution evaporated, weighed, ignited and reweighed. The loss in weight is taken to be "humus." The organic matter contains *ammonia* which comes from the ammonia solution used to extract it.

The various methods of analysis used differ; first, in the manner of decomposing the humates and dissolving the organic matter; and, second, in the methods for getting rid of the clay.

The method of the Association of Official Agricultural Chemists and that of Snyder differ in the procedure for decomposition of humates and solution of the humus. The differences, however, are only differences in manipulation. In the first named method the soil is extracted with acid on a funnel, while in Snyder's method the soil is washed by decantation in a flask or beaker with successive portions of acid. In the A. O. A. C. method the ammonia is added to the soil in one portion, while in Snyder's method the soil is extracted with successive portions of ammonia, and then made up to volume. Snyder's method is more nearly like the original Grandeau method, although Grandeau extracted on a funnel, and, as Houston has pointed out, did not secure complete extraction.

The clay is most difficult to remove. It is deflocculated by the ammonia, and goes in suspension. It contains combined water, which is lost on ignition and is, therefore, calculated as "humus." If the soil contain much humus, the clay may apparently settle in a comparatively short time, but if ammonium sulphate is then added to the apparently clear liquid, additional clay usually is precipitated. If little humus is present, clay may remain in suspension six months or longer. Such soils appear to contain much humus, yet really contain little. We have ammoniacal soil extracts in which clay and organic colloidal matter have been in suspension for over two years.

The revised methods of the Association of Official Chemists require filtration of the ammoniacal solution, and provide that "The filtrate must be perfectly clear." How the filtrate is to be made clear is not specified, and for many soils this provision must be disregarded, or else the method abandoned for these particular soils.

Peters and Averitt (Bulletin 126, Kentucky Agricultural Experiment Station) propose to correct for the clay present by subtracting 10 per cent of the weight of the "ash" from the loss on ignition. As they say, this method is uncertain, but better than none. We shall discuss this method further on.

F. K. Cameron filters through porous porcelain to remove the clay. The clay is removed, without doubt, but possibly some organic matter also

Mooers (Bulletin 78, Tennessee Experiment Station) proposes to evaporate the solution, and to take up the residue in ammonia. This removes a portion of the clay, and, by repeated evaporation and solution, it may be possible to remove more.

These methods have been subjected to more or less study by us, and the results of our work will be presented in the following pages.

COMPARISON OF A. O. A. C., AND SNYDER METHODS.

The A. O. A. C. method is described as follows:

Place 10 grams of the sample in a gooch crucible, extract with 1 per cent hydrochloric acid until the filtrate gives no precipitate with ammonium hydroxide and ammonium oxalate, and remove the acid by washing the soil with water. Wash the contents of the crucible (includthe asbestos filter) into a glass-stoppered cylinder, with 500 c.c. of 4 per cent ammonium hydroxide, and allow to remain, with occasional shaking, for twenty-four hours. During this time the cylinder is inclined as much as possible, without bringing the contents in contact with the stopper, thus allowing the soil to settle on the side of the cylinder and exposing a very large surface to the action of the ammonium hydroxide. Place the cylinder in a vertical position and leave for twelve hours, to allow the sediment to settle. Filter the supernatant liquid (the filtrate must be perfectly clear), evaporate an aliquot, dry at 100° C., and weigh. Then ignite the residue and again weigh. Calculate the humus from the difference in weights between the dried and ignited residues.

NOTE.—If the extraction of humus consists merely in the decomposition of the humates with production of free humic acid and union of this acid with ammonia, the action should take place immediately, and the extraction should not require so much time. It would appear that the ammonia is reacting with organic material in the soil to form soluble compounds in much the same way as the lignin of plant tissues acts with ammonia.

Snyder's method as used by us is described as follows:

Weigh 20 grams of the soil into a wide-mouthed cylindrical bottle of about 500 c.c. capacity, provided with ground-glass stopper. Add to the soil 200 c.c. of the 1 per cent hydrochloric acid. This should be added cautiously or carbonates may cause frothing over Stopper and shake from time to time during the day. Allow to stand over night and in the morning decant off the liquid into a filter when necessary. Add 200 c.c. of the hydrochloric acid to the residue, using a part of the acid to rinse back from paper to bottle any adhering soil. Shake the bottle from time to time and again allow to stand over night and again decant. Repeat this until all lime is extracted from the soil. Now wash the soil from the bottle on a filter paper and wash free of hydrochloric acid. When thoroughly washed, wash the soil back into the bottle, using 200 c.c. of 4 per cent ammonia. Shake every half hour during the day and allow to stand over night. In the morning decant the liquid off in bottles of 1 liter capacity. Add to the soil in the bottles another 200 c.c. of ammonia and again shake from time to time during the day and allow to stand over night and decant in the morning. Repeat the extracting with ammonia until all the humus is removed and the extract has little color. The extracts are now poured into a liter graduated flask, made up to the mark with distilled water, and allowed to settle for one week. Pour off eight to nine hundred c.c. of the solution without disturbing the sediment in the bottom. Wash the bottles and pour the solution back into them. The solution must be thoroughly shaken before each aliquot is removed for analysis. Weigh a platinum dish, pipette into it 100 c.c. of the extract and evaporate on a water bath to dryness, heat for three hours in a steam bath, cool in desiccator and weigh. This weight less the original weight of the dish gives total solids. Now ignite thoroughly and weigh. Report ash and loss on ignition.

TABLE 1.

Comparison of Snyder and A. O. A. C. Methods for Humus.

Soil No.		Loss on Ignition		Ash		Corrected Loss on Ignition	
		A.O.A.C.	Snyder	A.O.A.C.	Snyder	A.O.A.C.	Snyder
816	Per cent in soil...	.88	.70	1.63	2.92	.72	.41
817	Per cent in soil...	1.19	1.29	2.95	4.03	.90	.89
823	Per cent in soil...	1.86	2.39	5.87	9.69	1.28	1.42
829	Per cent in soil...	9.10	9.12	.60	1.52	9.04	8.97
326	Per cent in soil...	2.48	3.06	1.77	2.06	2.31	1.86
134	Per cent in soil...	1.97	2.06	3.18	5.89	1.65	1.47
	Average	2.91	3.10	2.66	4.35	2.65	2.50

Table 1 shows a comparison between Snyder's and the A. O. A. C. method. Both extractions were carried on at the same time and with the same solutions. Snyder's method gives a greater loss on ignition but the solution contains more clay (ash). If we correct by the Peter and Averitt method, by subtracting 10 per cent of the clay, we find that Snyder's method gives lower results than the A. O. A. C. method. The correction for ash is, as we have pointed out, an uncertain quantity.

We consider that the preference between the two methods must depend upon the ease of manipulation. The Association method has the advantage of bringing less clay into suspension, while it appears to extract as much organic matter, if not more.

With heavy clay soils the soil may gum during the extraction with acid per the A. O. A. C. method, and require a very long time for extraction and washing.

Correction by Peter-Averitt Method.—If no correction is made for the water lost on ignition by the clay in the ammonia suspension, the results of the analysis may be entirely wrong. Soils which really contain practically no humus may give a considerable amount of clay in suspension, and, by the method, appear to contain considerable amounts of humus.

In correcting by Peter's and Averitt's method, we subtract 10 per cent of the clay from the loss on ignition.

As pointed out by the chemists who proposed this method of correction, it is not a certain method, on account of the different amounts of water contained in the clay of different soils. Yet we are inclined to believe that this correction is better than no correction at all.

Analyses of a number of samples of clay precipitated by salts have been made by us, and the results are presented in tables in another portion of this bulletin. The amount of loss by ignition in these clays is somewhat variable. On the other hand, the clay contains organic matter. That is to say, there is no constant correction which can be made. Subtraction of a certain percentage of the clay is thus an uncertain correction, but still better than none at all.

This method will be referred to further in succeeding pages.

Filtration Through Unglazed Porcelain.—This method of purifying the dissolved organic matter was used by Cameron, though it was not proposed by him as a quantitative method. It has also been used by Fraps (see Bulletin 82, Texas Experiment Station, page 28).

In order to test this method, the following experiment was performed: One-half gram air-dry humic acid (prepared by us from soils) was dissolved in ammonia and made up to 250 c.c. and filtered through an unglazed porcelain filter, rejecting the first 50 or 75 c.c. Fifty c.c. of the filtrate was evaporated to dryness in a platinum dish, and loss on ignition and ash determined. The same determination was made on the original unfiltered solution. (See Table 2.)

There was an apparent loss of organic material in filtering the humus solution. That is to say, some of the organic matter apparently did not pass through the unglazed filter. In two instances, the apparent loss was very great, only half of the organic matter recovered.

In filtering these solutions, we used a Pasteur filter, which carried a bent glass tube reaching through a rubber stopper to the bottom of the filter. The humus solution came in contact only with glass and porcelain.

TABLE 2.

Filtration of Humus Solution Through Porcelain Tube.

Soil No.		Solids		Ash	
		Before	After	Before	After
852	Weight in grams	.1003	.0669	.0051	.0036
324	Weight in grams	.0999	.0983	.0059	.0202
134	Weight in grams	.0998	.0904	.0054	.0002
934	Weight in grams	.0988	.0453	.0030	.0022
742	Weight in grams	.0991	.0571	.0015	.0011
	Average	.0996	.0716	.0042	.0054

The water in the pores of the porcelain filter appears difficult of removal. For this reason, we tried another series of experiments.

One gram of humic acid (air-dry) was dissolved in ammonia and made up to 500 c.c. with 4 per cent ammonia. Fifty cubic centimeters were evaporated in a platinum dish to determine organic matter and ash. The remainder was filtered through an unglazed porcelain filter (as described above) into a measuring cylinder. The first two portions of 50 c.c. each were rejected. The third, fifth and seventh fifty cubic centimeters were measured with a pipette, and evaporated in a platinum dish as before. The results are presented in Table 3.

TABLE 3.

Successive Filtration of Humus Through Unglazed Porcelain.

	No. 852		No. 934	
	Organic	Ash	Organic	Ash
	Grams	Grams	Grams	Grams
Original	.0911	.0048	.0887	.0096
Third filtrate (50 c.c.)	.0847	.0015	.0799	.0102
Fifth filtrate	.0794	.0021	.0856	.0086
Seventh filtrate	.0842	.0011	.0878	.0103

It would appear that, even if care is taken to reject the first portions which come through the filter, the loss of organic matter may be 10 per cent of the quantity present. This method is very tedious, as it requires considerable time to filter such quantity of the liquid. There is also a possibility that some of the organic matter may not pass through the filter, as shown by the following experiment:

When a solution of gelatin is filtered through this unglazed porcelain filter, very little passes through:

Organic solution, in 25 c.c.	.0905 grams
First 25 c.c. filtered	.0082 grams
Third 25 c.c. filtered	.0023 grams

Gelatin was deposited on the outside of the tube. (See Bulletin 86, this Station.)

Precipitation of Clay with Salts.—Ammonium sulphate, potassium sulphate, and other salts, will coagulate the clay and cause it to precipitate. If a non-volatile salt is used, the solution may be evaporated to dryness and ignited. The difficulty with this method is that the salt may be decomposed or otherwise partly lost on ignition, thereby causing an increase in the percentage of volatile matter secured.

Table 4 gives the results of an experiment with three methods. Evaporation without filtration gives the highest results. These figures are not corrected by Peters and Averitt's method. Filtration through porcelain gives the lowest results. The greatest differences are with soils 98 and 137.

TABLE 4.

Humus by Three Methods.

Soil No.		Filtered, Pasteur Tube	Precipitated with 0.5 gm. Potassium Chloride	Unfiltered, settled 24 hours
95	Per cent	1.02	1.24	2.22
96	Per cent	.90	1.08	3.92
97	Per cent	1.12	....	5.27
98	Per cent	1.00	2.58	4.88
137	Per cent	1.30	2.20	3.04
138	Per cent	1.05	1.48	3.12
141	Per cent	1.25	1.54	2.70
142	Per cent	1.35	1.48	1.73
	Average	1.12	1.66	3.36

By Electrolysis to Remove Clay.—This method, tested in ammonia solution, did not appear promising. The clay was precipitated in some cases, but stayed out of suspension only as long as the current was on. We expect to test this method further.

By Evaporation for Removal of Clay.—Mooers proposes to evaporate the humus solution to dryness, take up with ammonia, and evaporate again if necessary. Finally, the dried residue is to be weighed, ignited, and weighed again.

In our studies, we took up the residue both with water, and with ammonia. The ammonia-humus which we prepared from precipitated humus is easily soluble in water, and it should not be necessary to dissolve it in ammonia. Table 5 shows a comparison between the method of direct evaporation, of one evaporation and solution in 4 per cent ammonia, and one evaporation and solution in water. These solutions are all from different samples of one soil.

The method of direct evaporation, as is seen, gives too high results. Evaporation and taking up with water brings much less clay into suspension again than evaporation and taking up with ammonia. In the evaporation, however, a considerable portion of the clay has been rendered insoluble, even if ammonia is used to take up these residues.

TABLE 5.

Comparison of Two Methods For Humus.

	A		B		C	
	Direct		Evaporated and taken up with Water		Evaporated and taken up with Ammonia	
	Loss on Ignition	Ash	Loss on Ignition	Ash	Loss on Ignition	Ash
1. Per cent of soil	*1.78*	*4.86*	*.94*	*.09*	*1.11*	*1.78*
2. Per cent of soil	1.89	4.15	1.29	.17	1.18	1.97
3. Per cent of soil	1.95	5.10	1.27	.14	1.42	1.66
4. Per cent of soil	2.09	4.89	1.20	.15	1.30	1.86
5. Per cent of soil	1.78	5.51	1.13	.14	1.24	1.65
6. Per cent of soil	1.83	4.73	1.19	.12	1.37	1.13
Average	1.89	4.87	1.17	.14	1.27	1.68

TABLE 6.

Results of Table 5 Corrected by Peters and Averitt Method.

	Loss on Ignition—Percentage.		
	A	B	C
	Direct	Evaporated Water	Evaporated Ammonia
1.	1.30	.94	.93
2.	1.47	1.27	.98
3.	1.44	1.26	1.15
4.	1.50	1.18	1.11
5.	1.23	1.12	1.08
6.	1.36	1.18	1.26
Average	1.38	1.16	1.08

Table 6 shows these results corrected by Peters and Averitt's method. The method of direct evaporation still gives higher results than the methods of evaporation and solution. Solution in water and solution in ammonia gives very nearly the same results, the latter being somewhat lower.

Table 7 contains a comparison between one evaporation and solution of the ammonium humate in (a) water and (b) in 1 per cent ammonia. These estimations were all made on the same solutions. The agreement between checks run at different times left much to be desired. It would appear that solution in 1 per cent ammonia is better than solution in water. The water does not appear to dissolve all the organic matter dried down with the clay.

The method of evaporation and solution in 1 per cent ammonia, filtering off the clay, appears to us to be the best method so far proposed for the estimation of the ammonia-soluble organic matter of the soil, although the agreement between checks run at different times was not always satisfactory.

TABLE 7.

Comparison of Method of Solution of Evaporated Humus in Water and in One Per Cent Ammonia.

Soil No.	Loss on Ignition		Ash		Loss on Ignition. Corrected by P. & A. Method	
	Water	Ammonia	Water	Ammonia	Water	Ammonia
876	1.70	2.04	.72	.76	1.63	1.96
939	1.84	2.28	.73	1.44	1.77	2.14
1203	.86	1.48	1.10	1.45	.75	1.34
1075	1.71	3.07	1.40	3.14	1.57	1.09
834	.98	1.70	.35	.26	.94	1.67
129	1.08	1.49	.73	.66	1.01	1.42
830	3.41	3.50	1.11	1.20	3.30	3.38
1582	3.18	3.67	.90	2.28	3.09	3.45
845	1.97	2.45	.66	4.09	1.90	2.04
851	1.68	2.25	.40	1.45	1.64	2.11
1202	1.53	2.94	1.61	3.33	1.37	2.61
844	1.15	1.52	1.35	.75	1.02	1.44
893	1.15	1.67	1.05	1.10	1.04	1.56
1595	1.24	1.67	1.20	1.30	1.12	1.54
829	1.20	1.86	.95	3.19	1.10	1.54
933	1.19	1.53	.90	2.49	1.10	1.28
934	1.62	2.58	.92	4.37	1.53	1.14
940	1.52	2.22	.65	....	1.46	....
941	1.33	1.80	1.40	3.12	1.19	1.49
Average	1.59	2.19	.95	1.96	1.50	1.84

Precipitation of the Humic Acid.—In this method, the humus is freed from clay by ammonium sulphate, the humic acid precipitated by hydrochloric acid, collected on a platinum gooch, dried, weighed, ignited, and weighed again. The loss on ignition is humic acid insoluble in water.

This method may be expected to give low results. The humic acid is, to some extent, soluble in water, as is usually shown by the brown color of the filtrate. The method can not be expected to be quantitative; at the same time, the amount of insoluble organic matter precipitated with acid is a matter of some significance.

The results of a number of analyses made by this method are given in Table 8.

The amount of humus precipitated is, in many cases, considerably less than the corrected amount by the Peters and Averitt method. In some cases, it is equal to that quantity. While, on the one hand, the loss on ignition residue, even when corrected, undoubtedly gives high results; on the other hand, the solubility of the organic precipitate will tend to give smaller results than the above method. These results are, however, of interest in showing the amount of organic matter which may be precipitated from the solution by acids. In terms of the uncorrected loss on ignition, the precipitated humic acid varies from 8 to 91 per cent. A part of this variation is, no doubt, due to the conditions under which the precipitation takes place, and a part to the differences in the nature of the material. The method of washing the precipitate also affects its quantity. We believe that the wash water should be slightly acid. We find that about two-thirds of the humus is precipitated.

TABLE 8.

Percentage of Precipitated Humus, Etc., in Soils.

Soil No.	Loss on Ignition		Precipitated Humus	Precipitated Humus Divided by Corrected Loss on Ignition (Per Cent)
	Direct (A)	Corrected by P. & A. Method		
816	.44	.34	.41	117
817	.75	.57	.59	104
823	2.48	.56	.19	34
829	1.74	1.39	1.45	105
326	2.08	1.73	1.35	78
134	1.10	.97	1.01	104
741	3.28	2.95	1.74	58
742	6.12	5.65	3.70	65
743	4.64	4.09	3.03	74
744	4.47	4.12	2.84	70
745	5.87	5.55	3.73	67
746	3.82	3.45	3.18	92
747	2.77	2.38	1.02	47
818	1.16	.81	.11	13
824	1.23	.67	.23	34
829	3.22	1.43	.58	41
843	3.85	2.71	.79	29
845	3.28	2.52	.95	38
851	1.98	1.46	.74	50
101	2.53	1.89	1.37	72
133	1.48	.97	1.30	130
180	1.24	.89	.62	69
182	2.03	1.39	1.14	82
332	3.98	3.82	1.34	35
Average	2.73	2.18	1.39	64

Effect of Extended Washing with Acid.—In this experiment, two portions of the soil were weighed out. One portion was washed with acid the usual number of times; the other was washed double this number. The washing with water and treatment with ammonia then followed, as with Snyder's method.

The results are shown in Table 9. The effect of the greater number of washings was to *increase* the quantity of clay which went into suspension. This increase varied from about 16 per cent to over 50 per cent in the three soils studied. The loss on ignition was slightly greater with the soils which were washed more times with acid, but when corrected by Peters and Averitt's method the humus is slightly less. The extended washing with acid appears to be unnecessary.

TABLE 9.

Effect of Washing With Acid.

Soil No.		Usual Washing			Washed twice as much		
		Ignition Loss	Ash	Corrected Ignition Loss	Ignition Loss	Ash	Corrected Ignition Loss
946	Percentage in soil............	4.88	6.28	4.25	5.26	9.96	4.26
947	Percentage in soil............	5.24	9.09	4.33	5.48	12.43	4.24
948	Percentage in soil............	6.36	6.74	5.66	6.39	8.84	5.51
	Average..........................	5.49	7.37	4.74	5.71	10.41	4.67

Effect of Various Strengths of Ammonia.—If the function of the ammonia is merely to form an ammonium salt of the humic acid and bring it into solution, then the quantity of ammonia used need only be the amount necessary for this change, allowing for the absorption of ammonia by the soil. If, however, the ammonia acts as a solvent for organic matter, or causes it to undergo some chemical change so that it enters into solution, then the quantity of ammonia used must have some effect upon the organic matter dissolved up to a certain point. It does not follow that the quantity of organic matter dissolved must increase as the quantity of ammonia used increases, without limit.

We conducted two series of experiments to test the effect of the strength of ammonia. In one series the strength of ammonia varied from 0.1 to .001 per cent. In the other series, the strength of the ammonia varied from 0.1 to 8.0 per cent. In both series, the soil was first treated with acid and washed with water, as in Snyder's method. The quantity of ammonia solution used was 1000 c.c. to 20 grams soil, and in other respects the method was the same as Snyder's method.

The results of the extraction with very dilute ammonia is shown in Table 10. When the weakest ammonia was used, 1 mg ammonia was brought in contact with 20 grams soil. In our other work, we have found that ammonia humate contains approximately 8 per cent ammonia. One milligram of ammonia would therefore dissolve about 12 milligrams humic acid, or about 0.6 per cent, under the condition stated above. There is, of course, a possibility that some traces of acid were retained by the sails, though they were washed thoroughly. The next grade of ammonia (0.01 per cent) could combine with 6 per cent humic acid in the soil, while 0.1 per cent ammonia would suffice for union with 60 per cent.

On examination of Table 10, we find that the weakest ammonia dissolves about 0.25 per cent humus. With the second strength of ammonia there is a difference between the soils. With two soils, the second strength ammonia dissolves more humic acid, but only from about one-fourth to one-eighth of the amount dissolved by the next strength. With the other two soils, there is little difference in the quantity of humus dissolved by the 0.1 and .01 per cent ammonia. It looks as though an error may have been made somewhere. It would appear, however, that an excess of ammonia over the quantity required to form ammonium humate, increases the amount of humus dissolved.

The quantity of phosphoric acid, and of clay, appears to increase with the strength of the ammonia, the former quite decidedly.

TABLE 10.

Extractions of Soils With Very Dilute Ammonia.

Soil No.		Loss on Ignition			Ash			Phosphoric Acid		
	Percentage of Ammonia in Solution	.1	.01	.001	.1	.01	.001	.1	.01	.001
946	Percentage in Soil	3.39	.45	.25	1.42	.66	.51	.05	.02	.01
949	Percentage in Soil	2.72	.73	.26	3.63	.89	.38	.05	.02	.01
324	Percentage in Soil	2.09	2.00	.25	13.52	15.83	.78	.03	.03	.01
940	Percentage in Soil	2.47	2.28	.23	14.16	11.74	.61	.02	.02	.01

It would appear from this work that an excess of ammonia over that required to form the ammonia salt is needed.

Table 11 shows the effect of ammonia of strength varying from 0.1 per cent to 8.0 per cent. As pointed out already, 0.1 per cent ammonia should suffice to convert humus in 60 per cent of the weight of soil into ammonium humate. In spite of this, increase of strength of ammonia from 0.1 to 0.5 increases the organic matter in solution, as a rule. This is shown both by the incorrected, and by the corrected, loss on ignition. There is also a slight average increase from 0.5 per cent ammonia to 1.0 per cent ammonia uncorrected. According to the corrected results, the percentage of humus increases with strength of solvent up to 8 per cent.

We consider these facts evidence that material goes into solution which is not "ammonium humate" but is merely organic matter soluble in the ammonia, or converted by it into soluble compounds.

The ash or clay taken up by the ammonia increases in quantity until it reaches a maximum, and then decreases. This maximum is not reached with the same strength of ammonia in all soils. With soils 946 the difference between maximum and minimum is about 1.1 per cent and the maximum is reached with 4.0 per cent ammonia. With soil 324, the maximum ash is 9.29 per cent and the minimum 0.82, the maximum being reached with 0.5 per cent ammonia. This strength ammonia also yields maximum results for soils 744 and 745.

From this work, 1 per cent ammonia is amply strong to dissolve the bulk of the ammonia-soluble organic matter. There *appears* no need to use an ammonia as strong as 4 per cent for soils as low in humus as those we have worked upon.

TABLE 11.

Extraction of Soils by Varying Strengths of Ammonia.

Soil No.		Loss on Ignition.						Ash.					
	Percentage of Ammonia	8	4	2	1	.5	.1	8	4	2	1	.5	.1
946	Percentage in Soil	4.62	4.68	4.19	4.37	4.21	3.62	2.17	2.64	2.35	2.25	1.54	1.56
949	Percentage in Soil	3.29	3.32	3.17	3.23	2.97	2.78	2.52	3.71	3.97	4.07	3.87	3.47
940	Percentage in Soil	2.49	2.91	2.81	2.81	3.34	2.94	6.15	10.35	10.65	11.78	12.50	12.79
324	Percentage in Soil	1.86	1.57	1.17	1.69	2.32	1.85	.82	2.74	3.70	5.51	9.29	8.15
744	Percentage in Soil	4.17	4.25	4.46	4.23	4.16	3.18	3.72	4.35	6.63	6.30	7.97	5.12
745	Percentage in Soil	5.55	5.26	5.08	5.25	4.66	3.38	3.65	4.78	5.19	6.79	7.01	5.36
	Average	3.66	3.53	3.48	3.69	3.61	2.96	3.17	4.80	5.41	6.11	7.03	6.16

Soil No.		Humus Corrected by Peters and Averitt Method.						Phosphoric Acid.					
	Percentage of Ammonia	8	4	2	1	.5	.1	8	4	2	1	.5	.1
946	Percentage in Soil	4.20	4.42	3.96	4.15	4.06	3.47	.08	.08	.07	.07	.07	.06
949	Percentage in Soil	3.04	2.95	2.77	2.83	2.58	2.41	.04	.05	.05	.05	.05	.05
940	Percentage in Soil	2.41	2.64	2.44	2.26	2.42	2.12	.02	.02	.02	.02	.01	.02
324	Percentage in Soil	1.25	.54	.11	.51	1.07	.57	.01	.01	.01	.02	.02	.02
744	Percentage in Soil	3.80	3.72	3.80	3.60	3.36	2.67	.09	.09	.09	.09	.09	.08
745	Percentage in Soil	5.79	4.82	4.56	4.57	4.33	3.12	.08	.10	.10	.09	.10	.10
	Average	3.41	3.18	2.96	2.98	2.97	2.39						

Houston and McBride (Bulletin 46, Indiana Experiment Station, 1893) studied the effect of the strength of the ammonia upon the humus as measured by the loss on ignition of the evaporated extract, using, however, soils much richer in humus or organic matter than those which we tested. The amount of "humus" increased with the strength of the ammonia up to the 8 per cent used. The difference between the 2 per cent and the 4 per cent ammonia extract was much greater than between the 4 per cent and 8 per cent. No determination was reported of the clay or "ash," so we are unable to judge how far the increase was due to more clay going into suspension. It appears from the results of these investigators, however, that the stronger ammonia will extract more organic matter than the weaker ammonia, from soils containing much organic matter. They also found the time of the digestion to be of influence when 2 per cent ammonia was used, especially on peat soils, but of not so much significance when the ammonia had a strength of 4 per cent. For example, with 2 per cent ammonia, 18.26 per cent "humus" was extracted from a peat soil in sixty hours, 24.40 per cent in seventy-two hours, and 24.62 in ninety-six hours. Four per cent ammonia extracted 27.6 per cent in sixty hours and 27.63 per cent in seventy-two hours. Ammonia is acting chemically upon the peat substance, producing soluble compounds—a manufacture of "humus," rather than merely a union of humic acid and ammonia.

A few results are presented by Houston and McBride which indicate that the temperature may have a decided effect on the quantity of humus extracted, especially from a peat soil. Digested thirty-six hours at 50° F., the peat soil yielded 20.70 per cent "humus," while at 80° F., it gave 28.70 per cent "humus." These results would also point to the manufacture of humus rather than simple combination between ammonia and humic acid, which should be largely independent of the temperature.

PART II—FORMATION OF AMMONIA-SOLUBLE ORGANIC MATTER IN THE SOIL.

According to Snyder (Bulletin 53, Minnesota Experiment Station), meat scraps, flour, and other organic matter produce ammonia-soluble organic matter when allowed to decay in the soil. Similar work is reported by Snyder in Bulletin 89 of the same experiment station with similar results. This work has been previously referred to in this bulletin (see page 9). Snyder did not correct for ammonia-soluble organic matter in the material he used. We have conducted further experiments to ascertain the effect of organic matter upon the humus and phosphoric acid of the soil.

METHOD OF WORK.

The soils selected for the experiment were first mixed thoroughly. Five hundred grams of the soil were weighed out into quart jars and mixed with 20 grams of the organic material to be studied. Water was added equal to one-third of the saturation capacity of the soil, the jar weighed and the weight marked thereon. The loosely-covered jars were stored in a dark basement and from time to time were weighed and the loss of weight restored by the addition of water. At the end of fourteen weeks, one set of the jars was taken and the contents dried and prepared for analysis. The other set was dried at the end of one year, the amount of water present being maintained in the meantime as before stated. Samples of the soils and of the organic materials which had been added to them were preserved, the latter being sterilized to prevent decay or fermentation.

The humus was determined by Snyder's method, as already described. No correction was made for the amount of ash brought into suspension. If there is any variation in the amount of ash, it will be referred to in discussing the experiment. As a rule, the amount of ash was fairly constant.

APPARENT FORMATION OF HUMUS.

If we compare the quantity of humus extracted from the soil containing the organic materials with the amount extracted from the original soil, we find that in all cases there has been an increase of humus, evidently due to the added materials. The results with one set of soils are given in Table 12. The addition of organic matter has apparently raised the quantity of humus in the soils .38 per cent after one year. The addition of cottonseed meal has raised it .44 per cent, blood .12 per cent and excrement .30 per cent.

As we will see later on, however, this increase is apparent rather than real.

TABLE 12.

Humus in Soils With Various Additions After One Year.

	Per Cent Humus (Uncorrected)
Original soil	1.29
Soil + meat	1.67
Soil + cottonseed meal	1.73
Soil + blood	1.41
Soil + excrement	1.59

THE ORGANIC MATTER CONTAINS AMMONIA-SOLUBLE MATERIAL.

The organic materials used in the experiment all contained matter soluble in ammonia. This is seen in Table 13. These results were secured by extracting the organic matter, first with acid, and then with ammonia, as in the extraction of humus from the soil. Five grams of substance were used and it was extracted once with 250 c.c. of hydrochloric acid, washed with water, and then extracted with 250 c.c. of 4 per cent ammonia. After ten hours the ammonia was decanted, 200 c.c. of ammonia added and allowed to stand five hours. The treatment was repeated with 200 c.c. more of ammonia and the filtrates made up to 1000 c.c. The results are presented in Table 13.

From 8 to 34 per cent ammonia-soluble organic matter was secured from these materials. If the ratio of material to solvent had been larger, as is the case in the extraction of soil, it is quite probable that a larger amount of organic material would have gone into solution. Nevertheless, these figures bring out clearly the fact that the organic matter introduced into the soil already contains ammonia-soluble materials. This fact should be taken into consideration before any statement as to the production of ammonia-soluble organic matter is made. Such a possibility was not considered in the experiment of Snyder which we have cited.

The amount of organic matter precipitated by acid was also estimated in this experiment. A much larger percentage of the humus of the soil is precipitated than is the case with the ammonia-soluble organic matter of these materials.

TABLE 13.

Material Soluble From Organic Materials in Ammonia After Extraction With Acid.

	Percentage of Material Used.					Organic Precipitate Per Cent of Total	Per Cent of Phosphoric Acid in Organic Precipitate
	Total	Organic	Ash	Phosphoric Acid	Organic Matter Precipitated by Acid		
Excrement No. 862	16.75	15.48	1.28	.23	5.88	38	.05
Excrement No. 898	15.98	14.68	1.30	.18	4.12	29	.05
Crude bat guano	10.08	8.64	1.44	.15	4.40	51	.06
Tankage	16.04	15.14	.90	.21	8.16	54	.06
Wheat bran	35.98	34.62	1.36	.41	14.66	42	.04
Cottonseed meal	11.30	10.80	.80	.11	6.26	58	.03

FORMATION OF HUMUS.

In order to correct for the ammonia-soluble organic matter contained in the organic material added to the soil, we prepared mixtures equivalent to the soil plus the organic matter at the beginning of the experiment and extracted these mixtures with acid and ammonia exactly as was done with the soil mixtures which had been allowed to stand fourteen weeks, and one year. The results of this work are presented in the tables. Table 14 shows the percentage of "humus" secured from the different soils and mixtures at the beginning of the experiment, after fourteen weeks and after one year. It should be repeated, however, that the mixtures were prepared from the original soil and the additions and extracted for "humus" at the same time under the same conditions as the mixtures which had been allowed to "humify." We believe that in this way we secured comparable analyses. A decrease of humus occurs. The ash is given in Table 15.

TABLE 14.

Humus in Soils and Mixtures After Various Intervals.

PERCENTAGE OF HUMUS.

Soil No.		Soil	Soil + Meat	Soil + Cottonseed Meal	Soil + Blood	Soil + Excrement
885	Original mixtures	1.36	2.29	2.81	1.68	1.71
	Mixtures after 14 weeks	1.30	1.68	1.83	1.76	1.45
	Mixtures after 1 year	1.29	1.67	1.73	1.41	1.59
895	Original mixtures	.94	2.11	2.27	1.42	1.22
	Mixtures after 14 weeks.	.96	1.29	1.20	1.40	1.21
	Mixtures after 1 year	.91	1.24	....	.97	1.09
958	Original mixtures	2.17	3.03	3.20	2.42	3.31
	Mixtures after 14 weeks.	2.40	2.96	2.68	2.25	2.03
	Mixtures after 1 year	2.17	2.65	2.08	2.24	2.18

The increase or decrease of the "humus" after humification is shown in Table 16. After allowing for the ammonia-soluble organic matter originally added to the soil, we find that in only four instances is there any apparent increase in "humus." There is on the other hand a decrease in humus in a great many of the other cases. The cottonseed meal mixture particularly showed a considerable decrease in its ammonia-soluble organic matter. Most of this decrease took place in the first fourteen weeks.

Table 17 shows the amount of humus in the various mixtures after evaporation and solution in water. The results are similar to the above, showing a decrease rather than an increase in humus, though in some cases an apparent increase occurs. Similar results are obtained by the method of filtration through porous porcelain (Table 18).

It appears from this experiment that there is no gain of ammonia-soluble organic matter in the soil. On the contrary, the ammonia-soluble material decreases.

TABLE 15.

Percentage of Ash of Humus in Soils and Mixtures After Various Intervals.

Soil No.		Soil	Soil + Meat	Soil + Cottonseed Meal	Soil + Blood	Soil + Excrement
885	Original mixtures	7.16	6.87	7.11	7.05	7.03
	Mixtures after 14 weeks.	7.28	6.76	6.60	6.98	6.70
	Mixtures after 1 year	6.85	5.80	6.33	6.11	6.26
895	Original mixtures	1.96	1.40	1.31	1.54	1.71
	Mixtures after 14 weeks.	1.69	.96	.87	.99	1.76
	Mixtures after 1 year	2.46	1.51	....	1.29	2.14
958	Original mixtures	2.67	2.20	2.46	1.73	2.37
	Mixtures after 14 weeks.	3.05	3.49	3.05	2.28	2.38
	Mixtures after 1 year	3.65	4.37	3.66	3.26	3.18

TABLE 16.

Gain or Loss of "Humus" in Soils With Various Mixtures.

PERCENTAGE IN SOIL.

Soil No.		Soil	Soil + Meat	Soil + Cotton seed Meal	Soil + Blood	Soil + Excrement
885	After 14 weeks. (Gain +, Loss –)	–.02	–.61	–.98	+.08	–.26
	After 1 year	–.01	–.01	–.10	–.35	+.14
895	After 14 weeks	+.02	–.82	–1.07	–.02	–.01
	After 1 year	–.03	–.05	....	–.043	–.12
958	After 14 weeks	+.23	–.07	–.52	–.17	–.18
	After 1 year	–.23	–.31	–.60	–.01	+.15

TABLE 17.

Percentage of Humus in Mixtures by Method of Evaporation and Solution in Water.

Soil No.		Soil	Soil + Meat	Soil + Cotton-seed Meal	Soil + Blood	Soil + Excrement
885	Original mixtures	.52	1.03	.97	.76	.66
	After 1 year	.50	.87	.63	.70	.61
895	Original mixtures	.60	1.35	1.34	.68	.73
	After 10 weeks	.43	.72	....	....	.72
	After 1 year	.46	.69	.73	.56	.69
958	Original mixtures	1.35	2.07	1.94	1.54	1.48
	After 1 year	1.31	1.63	1.63	1.64	1.71

TABLE 18.

Percentage of Humus After Filtration Through Porcelain Filters.

Additions.	Loss on Ignition.		Ash.	
	Original Mixture	After 14 Weeks	Original Mixture	After 14 Weeks
None	.92	.76	.35	.37
Meat	1.42	1.34	.23	.55
Cottonseed meal	1.98	1.08	.43	.40
Blood	1.03	1.14	.25	.25
Excrement	1.13	1.07	.40	.13

EFFECT OF AMOUNT OF WATER.

In this experiment two soils were mixed with excrement and maintained with different amounts of water for a period of fourteen weeks. The mixtures were then compared with the original soil as before described. The results are in Table 19.

By the method of direct ignition of the soil, the least loss of organic matter takes place with the soil having 77 per cent saturation. With soil 932, there is apparently a gain of humus in the saturated soil. By the method of evaporation and solution there is no apparent gain of humus with soil 932 and the greatest loss is when the smallest amount of water is present. The differences, however, are not great. The results with soil 914 are somewhat irregular.

TABLE 19.

Effect of Amount of Water on Per Cent of Humus in Soil.

	Direct Ignition		Evaporation and Solution	
	Soil No. 932	Soil No. 914	Soil No. 932	Soil No. 914
No water	1 .58	1 .25	.93	.68
22 per cent of capacity	1 .32	86	.85	.74
33 per cent of capacity	1 .37	.89	.88	.73
55 per cent of capacity	....	.96	.85	.73
77 per cent of capacity	1 .42	1 .29*	.95	.69
100 per cent of capacity	1 .77	.88	.93	.73

*This estimation contained over twice as much ash as the others.

EFFECT OF NATURE OF SOIL.

In this experiment, different soils were mixed with excrement and maintained at one-third their saturation capacity of water for fourteen weeks and for one year. The results are presented in Tables 20, 21 and 22.

With three of the soils, the loss of "humus" was very nearly the same, being about 0.20 on an average. With two of the soils, there was practically no loss of humus. Whether or not these differences in the power of a soil to oxidize or conserve organic matter would appear in other soils remains to be seen.

By the method of evaporation and solution, there is in all cases a loss of this "humus," being slight, however, with one soil. The range of error in this work is too large for us to be willing to say what differences, if any, exist in the power of soils to prevent the loss of ammonia-soluble organic matter. That is, we are unable to decide from this work whether the ammonia-soluble organic matter disappears more rapidly in some of these soils than others.

TABLE 20.

Effect of Nature of Soil—By Direct Evaporation.

Soil No.		Soil	Soil + Excrement	Soil + Excrement, 1 Year	Loss + or Gain
819	Percentage	.52	.78	.60	–.18
820	Percentage	.96	1.22	1.23	+.01
821	Percentage	.47	.98	.75	–.23
843	Percentage	2.68*	3.06†	.75‡	....
852	Percentage	2.60	2.95	2.75	–.20
859	Percentage	.24	.70	.68	–.02

*Ash 8.3 per cent.
†Ash 9.2 per cent.
‡Ash 2.2 per cent.

TABLE 21.

Effect of Nature of Soil—By Evaporation and Solution.

Soil No.		Soil	Soil + Excrement	Soil + Excrement, 1 Year	Gain + or Gain –
819	Percentage loss on Ignition	.40	.65	.56	–.09
820	Percentage loss on Ignition	.75	.87	.84	–.03
821	Percentage loss on Ignition	.74	1.10	.99	–.11
843	Percentage loss on Ignition	1.53	2.05	.93	....
852	Percentage loss on Ignition	1.02	1.23	1.07	–.16
859	Percentage loss on Ignition	.44	.73	.58	–.15

TABLE 22.

Effect of Nature of Soil on Ash of Humus.

Soil No.		Original Soil	Soil + Excrement	Soil + Excrement, After 1 Year
819	Per cent Ash	.21	1.20	1.12
820	Per cent Ash	2.35	2.60	2.10
821	Per cent Ash	3.19	3.00	2.58
843	Per cent Ash	8.34	9.24	2.18
852	Per cent Ash	10.10	9.26	7.98
859	Per cent Ash	.20	.28	1.00

EFFECT OF CHARACTER OF ORGANIC MATTER.

The data in the preceding Tables 14 and 15 allow us to compare the content and the loss of ammonia-soluble organic matter of blood, cottonseed meal, and some other materials. A series of experiments with other substances is presented in Table 23. The results of these experiments are not all in the same direction. It is evident that the extraction of ammonia-soluble material depends somewhat upon conditions.

When extracted alone, wheat bran (Table 13) gave the highest results, followed by excrement and tankage; cottonseed meal comes next, and bat guano last. The mixtures of soil and various substances gave the most soluble material to be from cottonseed meal; meat came next, blood next and excrement last. (See Table 14.) After the mixtures had humified a year, cottonseed meal loses its first place to the meat. That is, the ammonia-soluble organic matter of cottonseed meal decays more rapidly than that of meat. Blood and excrement come approximately in the same order. The results of the experiments with the three soils are different, and the preceding discussion refers to the average position.

Of the other mixtures which were studied, rice bran gave the most ammonia-soluble material to the fresh mixture, followed by tankage, bat guano and excrement, shorts, and corn chops, in the order named. The order is different after one year. Bat guano now comes first, rice bran second, wheat bran, corn chops and tankage third, and shorts last. This difference in order is due to difference of rate of decomposition of the ammonia-soluble material in the various materials when placed in the soil. The results are interesting, but the percentage of error in work on such amounts is too large for us to undertake to draw any general conclusions.

TABLE 23.

Effect of Nature of Organic Matter on Percentage Humus, Etc., in Soil.

	Loss on Ignition		Ash		Humus by Evaporation and Solution	
	Original Mixture	After 1 Year	Original Mixture	After 1 Year	Original Mixture	After 1 Year
Wheat Bran	1.21	.95	.73	1.25	1.21	.99
Shorts	1.17	.55	.73	1.37	1.19	.75
Bat Guano	1.22	1.16	1.12	.86	1.18	.95
Corn Chops	.91	.96	1.21	1.33	.66	.67
Rice Bran	1.48	1.12	1.68	1.02	1.11	1.13
Tankage	1.45	.93	1.22	.86	1.07	.76

PHOSPHORIC ACID.

The question of the formation of humus-phosphoric acid was also studied in connection with the above work, and the details will be published at some future time.

PART III—COMPOSITION AND PROPERTIES OF HUMIC ACID.

In this section, we deal with the composition and properties of the humic acid precipitates which we prepared from various soils.

METHODS OF PREPARATION.

Two methods were used.

1. *Ammonia Method.*—The soil was washed several times with 1 per cent hydrochloric acid to remove lime, washed with water, and digested with 4 per cent ammonia. After allowing the soil to settle for several days, the ammoniacal solution was drawn off and filtered. The soil was treated several times with the ammonia, the extracts combined, and ammonium sulphate (or chloride, in some cases), added to precipitate clay. The precipitated clay was allowed to settle, filtered off, and the humic acid precipitated by making the liquid slightly acid. It was washed thoroughly, and air-dried on clay or paper plates. The precipitate was very bulky when fresh, decreasing in volume considerably when dry. As the humic acid was not entirely free from ammonium salts, it was, in most cases, further purified. The dried material was finely powdered and digested with water, the water filtered off, the digestion repeated and the material finally washed on a filter. The purified humic acid was again dried, and ground.

The *clay* was washed thoroughly, and dried for analysis In most cases, it was purified as described above for humic acid.

The filtrate from the humic acid precipitate was not always colorless, but was sometimes of a dark-brown color. We precipitated this material from some of the solutions by means of metallic salts. The products will be referred to below.

2. *Phosphate-Soda Method.*—The soil was extracted with a solution containing 1 per cent caustic soda and 1 per cent sodium phosphate. The extracts were allowed to settle, and the dissolved material precipitated by means of a slight excess of acid. This method does not require so much manipulation as the ammonia method but the extraction of organic material is not nearly so complete, and the acid precipitation is not so complete either. Humus prepared by this method contained much ash. The results of the two methods will be compared later on (see page 40).

ACIDITY OF HUMIC ACID.

By Salt Water.—One gram humic acid was shaken with 250 c.c. of salt solution (Hopkins method for soil acidity) and filtered and 125 c.c. titrated with caustic soda and phenolphthalein, after boiling to expel carbon dioxide. The solution was acid.

One gram humic acid 1940 extracted with phosphate from soil 896 =2.6 c.c. N/10 NaOH.

One gram humic acid 1941 extracted with phosphate from soil 896 =2.4 c.c. N/10 NaOH.

One-half gram humic acid:
No. 1950 by ammonia=3.1 c.c. N/10 NaOH.
No. 1949 by ammonia=2.9 c.c. N/10 NaOH.
No. 940 by ammonia=2.9 c.c. N/10 NaOH.

No. 1940 by phosphate=1.9 c.c. N/10 NaOH.
No. 1937 by phosphate=0.7 c.c. N/10 NaOH.
No. 1805 by ammonia=3.3 c.c. N/10 NaOH.
No. 1941 by phosphate=1.1 c.c. N/10 NaOH.
No. 1939 by phosphate=2.2 c.c. N/10 NaOH.
No. 1948 by ammonia=3.3 c.c. N/10 NaOH.

Maximum is 6.6 c.c. N/10 caustic soda to 1 gram humic acid, which is equivalent to .01121 gram ammonia. As we have found the ammonia compound of humic acid to contain about 8 per cent of ammonia, it is evident that the acidity which goes into the salt solution is only about 12 to 15 per cent of the neutralizing power of the humic acid at the maximum.

It is evident that free humic acid has little power to decompose sodium chloride. That is to say, the acidity estimated by Hopkins' method would be much too low if due to humic acid.

By Carbonate of Lime.—Humic acid was boiled with carbonate of lime and water (previously boiled). The gases were passed into lime water. Carbon dioxide was rapidly evolved, showing that the humic acid has the power to decompose carbonates.

PRECIPITATION OF HUMUS AS SALTS.

The object of this work was to study the precipitation of humic acid by various bases.

Humic acids from soils Nos. 134, 934, and 324 (prepared by the ammonia method) were dissolved in ammonia and evaporated over sulphuric acid until the ammonia had disappeared, and then dissolved in water and made up to volume.

Aliquots corresponding to 0.5 gram humic acid were treated with various salts. Solutions of the salts were prepared and subjected to analysis to ascertain their strength. We assume that the calcium salt of the humate contained 7 per cent lime (CaO), and the equivalent amount of the other salt we term the theoretical quantity to form the humate. The assumption is based on analysis of salts (see page 35).

The results are as follows:

Alum.—One-fifth the theoretical caused a small precipitate, two-fifths a larger one, three-fifths precipitated all of No. 134, nearly all of No. 934, not so much of No. 924. Six-fifths precipitated the humic acid in the filtrate from the addition of three-fifths of the theory and left a colorless filtrate. Alum is an excellent precipitant.

Manganese Sulphate.—No precipitate with one-half of theoretical or with the full amount. With one and one-half times the theoretical, a slight precipitate occurred. With twice the theoretical, 934 and 134 nearly all precipitated, 324 not so much; thrice the theoretical completes the precipitation of No. 324. Manganese does not precipitate humic acid readily, and an excess must be present.

Zinc Sulphate.—With one-half of theory and one times theoretical, no precipitate was formed; with one and one-half times theoretical, a small precipitate. With twice theoretical, Nos. 934 and 134 nearly all precipitated; No. 324 much less but nearly all. With thrice theoretical, zinc precipitated all of No. 324. The behavior of zinc and manganese was very similar.

Mercuric Chloride.—A slight precipitate appeared when the addition had reached one and one-half the theoretical, but further additions, up to five times theoretical, produced no further precipitate with any of the humic acids.

Mercurous Nitrate.—No precipitate occurred with one-half times theoretical or the full theoretical. With one and one-half times theoretical, there was a good precipitate; much larger with Nos. 934 and 134 than with No. 324. The filtrates from Nos. 134 and 324 were completely precipitated and gave a colorless filtrate with double the theoretical. Filtrate from No. 934 was partly precipitated with two times, and with triple the theoretical it was completely precipitated.

Barium Chloride.—No precipitate with one-half times theoretical and one times theoretical. With one and one-half times theoretical partly precipitated. With double the theoretical, Nos. 934 and 134 completely precipitated; No. 324 nearly so.

Magnesium Chloride.—No precipitate up to one and one-half times theoretical, when a slight precipitate occurred. With double the theoretical, no further precipitation. With triple the theoretical, No. 934 precipitated partly; the others did not. With four times the theoretical, no further precipitation of No. 934 occurred, no precipitation of No. 134, some precipitation of No. 324, but not so much as No. 934. With five times theoretical, which was added only to 134, no precipitate was produced. Magnesium is not a good precipitant for humus. Magnesium humates are easily soluble.

Summary.—Humates do not behave towards precipitants like ordinary reactions, but, as a rule, require an excess of the reagent before precipitation occurs.

A difference is to be observed in the behavior of the humates from different soils. Humates from No. 324 was less easily precipitated with alum, manganese, and zinc, barium, and perhaps mercuric mercury, than the other humates.

The differences in the humates are most strikingly developed by the magnesium salts, humic acid from soil 134 forming practically no precipitate, while No. 324 required more of the reagent, and produced less precipitate than No. 934.

Further Studies of Precipitation.—A solution of ammonium humate was prepared by dissolving humic acid (prepared by ammonia from soil No. 939) and causing it to evaporate over sulphuric acid. It was then dissolved in water. Nearly equivalent amounts of solutions of calcium chloride, barium chloride, magnesium chloride and copper sulphate, were added. The strength of the solutions was determined by analysis. The results are shown in the table:

TABLE 24.

	Baryta (BaO)	Lime (CaO)	Magnesia (MgO)	Copper (CuO)
First Addition	.0948	.0353	.0268	.0494
Precipitate	None.	None.	None.	Complete.
Second Addition	.0948	.0353	.0268	....
Precipitate	Heavy.	Heavy.	None.	....
Third Addition	....	....	.0268	....
Precipitate	....	....	Same.	....
Fourth Addition	....	....	.0268	....
Precipitate	....	....	Not complete.	....

The barium salt appears to be the least soluble, the calcium salt next, magnesium most. The solution above the barium precipitate is colorless; that above the calcium salt is brown.

In another series of experiments, conducted at the same time as above, a small amount of ammonia was present. The only difference apparent was that the copper salt was not all precipitated by the first addition, and the solution above all the other precipitates was darker.

It is a striking fact that the addition of a lime salt containing sufficient lime to form the calcium salt containing 7 per cent lime, should not cause any precipitate at all. The lime salt, when formed, is not easily soluble in water. It would appear that the precipitation of humic acid by lime is not merely caused by the formation of an insoluble salt, but that an excess of lime must be present before this salt can be formed. The same applies to the barium salt. The magnesium salt is easily soluble in water, in many cases.

In another experiment, a solution containing 3 grams humic acid in about four liters of water required 140 c.c. of calcium chloride (of the strength stated above) to precipitate it. The precipitate was allowed to settle, decanted, filtered, and washed, and again suspended in water. It was reprecipitated by 25 c.c. of calcium chloride solution.

COMPOSITION OF HUMIC SALTS.

Salts of various bases with "humic acid" have been prepared by us by precipitating ammonium humate with a slight excess of the salt. The ammonium humate was prepared by dissolving humic acid in ammonia, and evaporating the solution over sulphuric acid until all odor of ammonia had disappeared. Sometimes we allowed the evaporation to proceed to complete dryness.

In Table 25, we show the composition of some of the humates. The combining weight of the humic radical, if univalent, would appear to vary from about 228 to 327, calculated from the composition of the ammonia salt. We have calculated the theoretical composition of the other salts, from the combining weight given in the table, and inserted it in the table. The humic acid is not, of course a definite chemical compound, but is a mixture of various bodies. It is possible that some of these bodies may be precipitated by some of the bases, and others not so well. There is also the possibility of the formation of basic salts, or even of double salts with ammonia and the precipitating metal.

TABLE 25.

Salts of Humic Acids.

	Soil No. 946	Soil No. 947	Soil No. 948	Soil No. 949	Soil No. 852	Soil No. 939	Soil No. 934
Percentage N. as Ammonia in Ammonia Salts	5.45	5.78	4.93	4.07	5.35	....	....
Equivalent weight of Organic Acid Radical Body on Ammonia Salt	240	228	271	327	244	234*	250*
Theoretical percentage of CaO in Salt based on Equivalent Weight	10.5	10.7	9.4	7.9	10.4	....	....
CaO found in CaO Salt	10.5	9.3	10.3	8.2	7.3	10.8	10.4
Theoretical percentage of BaO in BaO Salt based on Equivalent Weight.	24.1	25.1	22.1	19.0	....	24.6	23.4
BaO found in BaO Salt	21.5	20.8	19.4	16.5	....	27.9	21.9
Theoretical percentage MgO in MgO Salt	....	....	....	....	7.5	7.9	7.4
MgO found in MgO Salt	....	....	....	....	4.5	7.6	7.4

*Based on the lime salt.

The calcium salts contain more or less lime than the calculated, approaching quite closely to it in several cases. The barium found is considerably lower than the calculated. The magnesia is, in one case, much lower than the calculated; in the other, quite near to it.

According to these results, the "humic acid" is an acid body, and has a fairly definite combining weight.

The analyses referred to above were calculated to a moisture and ash-free basis. That is to say, the salts always contained ash other than the main precipitant, but our calculations are so made that the salt consists only of organic matter and the precipitant. It is quite possible that the other ash constituents affect the composition of the salt, or the combining value of the humic acid.

Table 26 shows the analysis of the lime salts of two humic acids. It also shows the copper oxide found in the copper salt.

TABLE 26.

Analysis of Salts.

	939 Lime Salt	852 Lime Salt	939 Copper Salt	324 First Alum Precipitate	324 Second Alum Precipitate
Moisture		11.16			
Ash	12.80	9.40			
Iron and Alumina Oxides	1.90	1.44		1.06	10.12
Lime	10.80	6.28			
Copper in Copper Salt			17.4		
Silica		1.08			

SOLUBILITY OF HUMIC SALTS.

Salts of humic acid from soil No. 934, ammonia method, precipitated, washed and air-dried, were suspended in cold water and allowed to stand twenty-four hours. At the end of that time, the residue was filtered off, and 50 c.c. of the filtrate evaporated to dryness, dried, weighed, ignited, and weighed again.

The insoluble residue was heated with water three hours in a boiling water bath with a reflux condenser, filtered, and 50 c.c. evaporated as before. The residue was extracted a third time.

The results are presented in the table.

TABLE 27.

Solubility of Humic Salts.

	Grams Dissolved in 50 c.c.					
	Soluble in Cold Water		Soluble in Hot Water			
			I.		II.	
	Organic	Ash	Organic	Ash	Organic	Ash
Magnesium Salt	.0325	.0126	.0339	.0096	.0272	.0085
Calcium Salt	.0019	.0027	.0065	.0029	.0096	.0083
Barium Salt	.0033	.0001	.0055	.0019	.0078	.0073

The solution from the magnesium salt had an intense color According to this experiment, the magnesium salt is the most soluble, the calcium and barium salts much less so.

EFFECT OF AMMONIA ON SOLUBILITY OF CALCIUM SALT.

This experiment was similar to the above, excepting that calcium carbonate, magnesium carbonate, and ammonia, were added to separate suspensions of calcium humate in water. The amount of ammonia used was 5 c.c. of N/10 ammonia to 100 c.c. solution.

The ammonia solution was most highly colored, the solution with no addition next, the magnesium carbonate next, and calcium carbonate least. In spite of this, however, the calcium carbonate and magnesium carbonate has no effect upon the calcium humate. The results of the experiment are as follows:

TABLE 28.

Effect of Additions on Solubility of Calcium Humate.

	Organic Matter in 50 c.c.
No addition	.0075
Magnesium carbonate	.0078
Calcium carbonate	.0082
Ammonia	.0192

Addition of ammonia, therefore, increased the solubility of calcium humate decidedly.

EFFECT OF CARBONATES OF LIME AND MAGNESIA ON SOLUBILITY OF HUMIC ACID.

Fresh, moist, humic acid was placed in test tubes with 15 or 20 c.c. water. One tube received no addition, a second some carbonate of lime, a third some carbonate of magnesia, a fourth carbonate of lime and caustic soda and a fifth carbonate of magnesia and caustic soda. The tubes stood twenty-four hours, being shaken occasionally. At the end of that time, the results were as follows:

TABLE 29.

Solubility of Humic Acid.

	No. 946	No. 947	No. 948	No. 949
Water alone	Light straw color	Wine color	Light straw	Light straw
Carbonate of Lime	Very light straw	Light straw	Darker straw	Light straw
Carbonate of Magnesia	Very dark brown	Black	Dark straw	Straw
Carbonate of Lime Caustic Sod	Black	Black	Black	Black
Carbonate of Magnesia, Carbonate of Soda	Black	Black	Black	Black

The color of the solution is an indication of the solubility of the humic acid.

Carbonate of lime decreases the solubility of the humic acid, but does not render it completely insoluble. Carbonate of magnesia causes the humic acid to be more soluble than in water. The addition of caustic soda increases the amount of humate in solution, even though carbonates of lime or magnesia are present.

HYDROLYSIS OF HUMIC ACID.

One-half gram humic acid from soil No. 852 prepared by ammonia was placed in a flask with 100 c.c. of 1 per cent hydrochloric acid, heated in a boiling water bath for five hours, neutralized, and the reducing sugars estimated. Reducing sugars found were 2.25 per cent. Another portion of one-half gram humic acid from soil No. 852 prepared by ammonia was dissolved in 10 c.c. of concentrated sulphuric acid, diluted to 200 c.c. and heated five hours in boiling-water bath, neutralized, etc., as before. Reducing sugars found were 2.40 per cent.

DIFFUSION OF HUMUS.

It has been claimed by some investigators that humus will not pass through parchments. This is denied by others. Experiments were made to test this matter.

First Experiment.—One-half gram of humus is dissolved in as small a quantity of water and ammonia is possible, placed in a diffusion shell (CS&S) and this is supported in a Jena glass vessel containing 500 c.c. of 4 per cent ammonia. After twenty-four hours the ammonia is evaporated in a platinum dish, dried, weighed, ignited and weighed. This is repeated with further quantities of ammonia (four diffusions made).

That the humus diffused was evident from the dark color of the solution outside the shell. Approximately 10 per cent of the ammonium humate passed through the capsule in four diffusions. (See Table 30.)

TABLE 30.

Diffusion of Humus.

	Total	Loss on Ignition	Ash	Insoluble Ash
747 Percentage diffused in first 24 hours	6.60	5.84	.76	.48
Second 24 hours	2.70	2.44	.26	.06
Third 24 hours	1.24	1.52	.22	.06
Fourth 24 hours	1.94	1.34	.06	.54–

Second Experiment.—One gram humus is dissolved in as small a quantity of water and ammonia as possible, placed in a diffusion shell, and supported there in a vessel of Jena glass containing 500 c.c. of 4 per cent ammonia. It is protected carefully from acid fumes. After twenty-four hours the ammonia is evaporated in platinum dish and ash and loss on ignition determined. Again, 500 c.c. ammonia is placed in the vessel, allowed to diffuse, and so on, for eight successive diffusions. A blank determination is made, using the same ammonia and a diffusion shell.

Results.—The results are presented in Table 31. The humus preparation appears to contain easily diffusible matter. After the latter has separated out, which takes two or three diffusions, the residual humus diffuses at the nearly constant rate of 1 to 2 per cent of the humic acid. The diffused solution was colored. Extraction with alcohol removes the easily diffusible material. The figures in the table are *not* corrected for the blank.

TABLE 31.

Diffusion of Humus.

	Blank	910 Original	910 Extracted Alcohol	894	934
First Diffusion.—Loss on Ignition in grams	.0061	.1620	.0123	.0486	.0519
Ash in grams	.0146	.0182	.0150	.0171	.0146
Second Diffusion.—Loss on Ignition in grams	.0039	.0240	.0099	.0146	.0275
Ash in grams	.0138	.0131	.0117	.0128	.0416
Third Diffusion.—Loss on Ignition in grams	.0055	.0168	.0107	.0127	.0364
Ash in grams	.0109	.0099	.0121	.0108	.0216
Fourth Diffusion.—Loss on Ignition in grams	.0041	.0135	.0069	.0269	.0300
Ash in grams	.0042	.0035	.0053	.0044	.0193
Fifth Diffusion.—Loss on Ignition in grams	.0062	.0102	.0084	.0252	.0192
Ash in grams	.0026	.0027	.0039	.0034	.0128
Sixth Diffusion—Loss on Ignition in grams	.0050	.0099	.0071	.0256	.0137
Ash in grams	.0036	.0039	.0035	.0030	.0077
Seventh Diffusion.—Loss on Ignition in grams	.0073	.0125	.0096	.0239	.0140
Ash in grams	.0043	.0045	.0061	.0044	.0098
Eighth Diffusion.—Loss on Ignition in grams	.0045	.0125	.0064	.0245	....
Ash in grams	.0070	.0066	.0060	.0084	....

COMPOSITION OF HUMIC ACID.

Table 32 contains estimation of water, ash and phosphoric acid in a number of humic precipitates. Oxides of iron and alumina, lime, and magnesia, are also estimated in a few of the precipitates.

The quantity of ash varies from 1.53 to 8.01—average 3.29 per cent. This is considerably less than when the clay is not precipitated previous to separating the humic acid.

The quantity of water is a matter of little consequence.

The percentage of phosphoric acid varies from 0.13 to 0.54 with an average of 0.30. This is a comparatively small amount of phosphoric acid.

Part descriptions of the soils from which these preparations were made are as follows: Analyses and full description of the Texas soils have been printed in Bulletins 99 and 125 of this Station.

DESCRIPTION OF SOILS USED FOR HUMIC ACID.

No. 134—San Antonio clay loam, San Antonio, Texas.
No. 324—Houston black clay, San Marcos, Texas.
No. 882—Wabash clay, subsoil, 10-36″, two and one-half miles northwest, Stockdale, Texas.
No. 896—Norfolk fine sandy loam, Lufkin, Texas.
No. 910—Houston black clay, 0-10″, Elgin, Texas.
No. 915—Houston black clay, 10-36″, Cooper, Texas.
No. 934—Wabash clay, 0-10″, Stockdale, Texas.
No. 939—Houston black clay, 0-10″, Cooper, Texas.
No. 946—Soil from virgin prairie, North Dakota.
No. 947—Soil from alfalfa field, North Dakota.
No. 948—Soil from a garden, North Dakota.
No. 949—Soil from old field; substation at Edgley, North Dakota.
No. 1739—Soil from Arroyo Grande, California.
No. 1740—Soil from Berkeley, California.
No. 895—Lufkin fine sand, Lufkin, Texas.
No. 982—Cameron clay, subsoil, Brownsville, Texas.
No. 896—Norfolk fine sand, Lufkin, Texas.
No. 882—Wabash clay, Stockdale, Texas.

TABLE 32.

Composition of Humic Acid.

Soil No.		Water	Ash	Loss on Ignition	Phosphoric Acid	Oxides of Iron and Alumina	Lime	Magnesia
742	Humic acid	11.60	7.16	81.24	.54	1.40	.19	.17
743	Humic acid	15.16	2.54	82.30	.27	1.00	Trace.	Trace.
743	Humic acid purified by lime	11.91	2.35	85.74	.32	.42	Trace.	Trace.
127	Humic acid	11.26	2.54	86.20	.28			
133	Humic acid	14.42	2.72	82.86	.32			
180	Humic acid	12.07	3.07	84.86	.45			
182	Humic acid	17.50	3.10	79.60	.36			
332	Humic acid	10.88	1.53	87.59	.23			
134	Humic acid	4.63	5.50	89.87	.17			
324	Humic acid	12.20	2.05	85.75	.24			
934	Humic acid	9.51	8.01	82.48	.13			
852	Humic acid	10.04	2.48	87.48				
1505	Humic acid	10.92	1.40	87.70				
1506	Humic acid	12.79	1.60	85.76				
	Average	11.77	3.29	84.96	.30			

COMPOSITION OF HUMIC ACIDS PURIFIED BY AMMONIA.

Table 33 shows the chemical composition of a number of humic acids extracted by ammonia. In some instances more complete analyses were not made on account of the small quantity of material which we had secured. All these products were purified by shaking the dried humic acid with water several times excepting Nos. 852, 127, 910 and the sample of No. 134, for which nitrogen is given. All the samples that are not purified contain appreciable quantities of ammonia. This ammonia was expelled by boiling the humic acid with magnesium oxide and water, and collected in standard acid. Other preparations of humic acid not purified also contained ammonia. (See Table 34.) These results are on air-dry samples.

TABLE 33.

Percentage Composition of Purified Humic Acids Extracted by Ammonia—Dry Basis.

		Carbon	Hydrogen	Nitrogen	Ash
1950	Purified from soil No. 934	54.13	3.27	4.31	8.85
1948	Purified from soil No. 324	55.39	4.77	6.22	2.64
852	Purified from soil No. 852	63.58	5.45	4.94x	2.75
1949	Purified from soil No. 134	55.15	3.48	....	6.11
1808	Purified from soil No. 949	44.09	3.14	3.98†	....
1805	Purified from soil No. 946	56.45	3.33	5.38*	1.57
127	Not Purified from soil No. 127	56.04	4.18	....	2.94
1807	Purified from soil No. 948	54.32	3.32	4.58*	15.74
1806	Purified from soil No. 947	55.63	4.15	5.44	1.94
910	Not Purified from soil No. 910	....	....	4.14	....
134	Not Purified from soil No. 134	....	....	6.24‡	....

x.96 Per cent N. as Ammonia present. Nitrogen on air-dry substance.
†.62 Per cent N. as Ammonia present. Nitrogen on air-dry substance.
*Air-dry.
‡2.96 Per cent nitrogen as ammonia.

TABLE 34.

Nitrogen and Ammonia in Humic Acids—Not Purified.

	Per Cent	
	Total Nitrogen	Nitrogen as Ammonia
From Soil No. 849	5.80	2.18
From Soil No. 934	4.96	1.72
From Soil No. 324	5.32	1.12
From Soil No. 745-7	5.16	....

It is evident that the humic acid may contain ammonium salts, unless special care is taken in purifying it. This is further evident when we study the alcoholic extract of the humic acid. (See page 44.)

Examination of the composition of these humic acids, prepared by first precipitating the clay from the ammonia solution, shows that we have succeeded in securing a product with a comparatively low ash.

The carbon content of these humic acids varies from 44.09 to 63.58. The majority of the samples contain between 54.13 and 56.45 per cent carbon. The two soils which contained humic acids not within these limits are as follows:

Soil No. 949, soil from an old field, North Dakota, humic acid low in carbon and also low in nitrogen.

Soil No. 852, soil from a rice field, Texas, humic acid high in carbon, moderate in nitrogen. The soils yielding humus a little high in nitrogen both came from the same locality in the western part of the State. Preparations of humic acid from other soils in the same section are not high in nitrogen.

The following table shows the composition of some humic acids obtained by Eggertz and by Snyder. Eggertz's work is based on thirteen analyses, Snyder's on four. The table shows the variation in their composition. Our samples average higher in carbon content than these samples. The content of nitrogen may probably be the same as the samples analyzed by Eggertz, though none of our samples run as low as his. Snyder's preparations all contain considerably more nitrogen than ours.

PERCENTAGE COMPOSITION OF HUMIC ACIDS.

	Eggertz	Snyder
Carbon	40.8 to 56.2	44 to 50
Hydrogen	4.3 to 6.6	3 to 6
Oxygen	25.1 to 38.0	28 to 35
Nitrogen	2.6 to 6.4	6.5 to 10
Silica	.4 to 10.5	
Phosphorus	.15 to 7.6	
Sulphur	.6 to 2.1	
Alumina and Oxide of Iron	.4 to 3.9	
Ash		4 to 12

HUMIC ACID EXTRACTED BY PHOSPHATE.

This method of extraction has already been described (see page 31). The composition of the products is shown in Table 35. This method does not give as good a product as our ammonia method, as the ash content of the precipitate is high. The carbon and hydrogen and nitrogen are made upon the material dried at 100°, the other estimations on the air-dry substance. These materials were all purified by shaking with water (5 c.c. water per gram of substance), then filtered and washed.

The humic acid prepared by the soda-phosphate method contains considerable ash, or clay. The presence of the ash introduces an error since it undoubtedly contains some water of hydration. For the purposes of comparison, we have calculated the analyses to ash-free material. On account of the water in the clay, we did not think it worth while to calculate the percentages of hydrogen. For the same reason, the carbon and nitrogen as calculated are probably a little low, as a portion of the ash-free material is water belonging to the clay. The amount of this error would increase with the quantity of the ash and also depend upon the nature of the clay. We have at present no means of introducing a correction for this water of clay. I do not consider the Peter and Averitt method of correction at all applicable to these preparations. The low carbon content is in a preparation from a soil near Lufkin, Texas. The low nitrogen is in the same soil. The high carbon is in the sample of Norfolk fine sandy loam from Lufkin.

With the exceptions of soil No. 895 (preparation No. 1937), the nitrogen content of these preparations is remarkably similar. The soils from California were secured for the express purpose of studying the nitrogen content of the humus, and the preparation by means of soda-phosphate was used so that there could be no question of ammonia absorbed by the preparations. The California soils, however, do not give us humic preparations containing any more nitrogen than our Texas soils. Two of these Texas soils came from arid or semi-arid sections, it is true, but the other one came from a humid part of the State.

The samples of California soils were sent to us by Dr. R. H. Loughridge, Berkeley, California, for which we hereby express our appreciation. (Numbers 1739 and 1740.)

TABLE 35.

Percentage Composition of Humic Acid by Soda Phosphate.

	1937 From Soil No. 895	1938 From Soil No. 982	1940 From Soil No. 896	1941 From Soil No. 882	2382 From Soil No. 1739	2383 From Soil No. 1740
Carbon on water-free substance	39.53	25.43	35.85	15.53	48.43	39.10
Hydrogen on water-free substance	3.51	3.82	3.72	1.76	2.96	2.36
Nitrogen on water-free substance	2.81	2.24	2.83	1.27	3.59	3.33
In air-dry substance—						
Ash	13.29	49.32	33.71	60.26	10.69	19.22
Water	10.67	8.02	10.64	11.59	9.70	11.10
Loss on Ignition	76.04	42.66	55.65	28.15	79.63	69.68
Phosphoric Acid	.....	.....	.28	.34	.....	.....
Insoluble Ash	.....	.....	25.09	47.29	.....	.....
Oxides of Iron and Alumina	.....	.....	5.05	8.11	.....	.....
Lime	.....	.....	.01	.02	.....	.....
Magnesia	.....	.....	.19	.75	.....	.....
On Ash-free material—						
Carbon	52.00	59.60	64.40	53.20	60.80	56.90
Nitrogen	3.69	5.25	5.08	4.51	4.51	4.78

Humic Acid from Copper Precipitate.—After precipitating humic acid from ammonia with acid there remains in solution some organic matter which can be precipitated by copper sulphate. The precipitated material was extracted with acid to remove copper, dried and subjected to analysis. The quantity secured was small. Product No. 1816 came from soil No. 946 and was almost white. No. 1817 was black, from soil No. 947. No. 1818 was brown, from soil No. 949. The analyses show that these precipitations are largely inorganic. The ash was found, on analysis, to consist largely of silica, though alumina and iron were also present.

Calculated to ash-free material, these precipitations contained the following amounts of carbon:

No. 1816, 27.0 per cent carbon.
No. 1817, 48.9 per cent carbon.
No. 1818, 53.8 per cent carbon.

TABLE 36.

Composition of Humic Acids from Copper Precipitate—Water Free.

No.		Carbon	Hydrogen	Nitrogen	Ash
1818	Brown Humic Acid from Copper Precipitate	9.09	2.11	....	*83.3*
1817	Black Humic Acid from Copper Precipitate	9.15	2.01	.60	81.3
1816	White Humic Acid from Copper *Precipitate*	*2.70*	*1.44*	....	*90.1*

ALCOHOL-SOLUBLE HUMUS PRODUCT.

A small percentage of the humus is soluble in alcohol. In order to separate it, the dried humic acid was extracted with boiling alcohol several times, the filtrate evaporated to dryness, pulverized, and extracted with water to remove ammonia salts which were present when the humic acid has not previously been purified. The analyses are presented in the following table. The quantity of alcoholic extract is comparatively small.

TABLE 37.

Percentage Composition of Alcoholic Extract.

No.		Oven Dried		Nitrogen (Air-dried)
		Carbon	Hydrogen	
1811	From Humus of Soil No. 934	63.72	6.33	....
1812	From Humus of Soil No. 915	59.38	4.18	....
1813	From Humus of Soil No. 910	66.50	6.73	....
1815	Residue from Extraction of Humus, Soil No. 910	59.15	4.08	4.20
	Second alcoholic extract of soil No. 910	....	....	8.21
	Second extract, soil No. 915	....	....	7.40
	Third extract, soil No. 915	....	....	6.80

The second alcoholic extracts were not purified as described above, as we assumed that all the ammonia had been extracted by the first treatment.

The products of the alcoholic extraction are richer in carbon than the original humus. The first extraction of the unpurified humic acid consists largely of ammonium salts.

THE CLAY OR ASH ASSOCIATED WITH HUMUS.

The mineral matter associated with the humus is largely clay. As we have seen, the clay can be easily coagulated by the addition of ammonium sulphate in the proportion of 5 grams per liter. Ammonium chloride, potassium sulphate, and other salts can be used also. The coagulated clay settles readily, and the clear liquid can be easily filtered off.

There is, of course, a possibility that along with the clay organic matter may be precipitated.

DISTRIBUTION OF CLAY OR ASH.

The amounts of clay or ash found with the coagulated clay, the clay precipitated with the humic acid, and the ash which remains in solution, is shown for a number of soils in the following table. The solutions were prepared by Snyder's method, as already described. (See page 11.) Where the same number appears twice, the results are not duplicates, but are made on different solutions, and the amount of clay which goes into suspension can not be expected to remain constant. (See Table 38.)

TABLE 38.

Distribution of Ash in Humus Products.

Soil No	Percentage on Soil		
	Total Ash	Clay Ash	Humic Ash
741	3.35	2.85	.06
742	4.74	3.18	.10
743	5.48	4.71	.06
744	3.53	2.55	.05
745	3.16	2.31	.18
746	2.66	.07	.71
101	6.46	6.05	....
127	1.92	1.55	....
133	5.08	4.92	....
180	1.60	.97	....
182	6.44	6.12	....
332	1.53	.91	....
818	3.46	3.20	....
824	5.63	4.86	....
829	7.89	6.92	....
843	11.37	8.58	....
845	7.56	5.75	....
851	2.66	1.82	....

A large portion of the ash material is precipitated as the clay, but some remains in solution, and some precipitates with the humic acid. When organic materials are treated with acid and then with ammonia, some of the ash is precipitated with the organic precipitate thrown down by acids.

COMPOSITION OF THE CLAY PRECIPITATE.

A number of samples of the clay precipitate were prepared in the course of our humus work, and have been subjected to analysis. Table 39 shows the partial analyses of some of these precipitates.

The loss on ignition varies from 8.41 to 16.34 per cent with an average of 14.40. According to these figures, the method of subtracting 10 per cent of the ash would not be correct, as it is too low, rather than too high.

Analyses of the clay precipitates, however, show them to contain carbon. The results of these analyses are presented in Table 40. A portion, at least, of the loss on ignition is due to organic matter in the clay precipitate. Correcting for the humus in the clay precipitate, we find a loss on ignition of less than 10 per cent.

After we correct for the organic matter in the clay, we find the loss on ignition to be very variable—from 1.77 to 13.04 per cent. The average loss is 8.38. If a correction is to be made, therefore, we think that it would be better to make a correction of 8 per cent of the ash, rather than the 10 per cent proposed by Peters and Averitt. Even this correction is a little high, because the clay contains salts of ammonia.

TABLE 39.

Percentage Composition of Clay Precipitates.

Number	Loss on Ignition	Water	Ash	Soluble Ash	Insoluble Ash	Phosphoric Acid
101	16.34	3.68	79.98	13.78	66.20	.06
127	15.54	6.30	78.16	15.10	63.06	.03
133	14.49	6.37	79.14	9.72	69.42	....
180	16.07	3.17	80.76	19.87	60.89	.04
182	15.70	5.03	79.27	6.88	72.36	.06
332	18.31	5.14	76.52	7.14	69.38	.04
742	20.48	8.48	71.04	20.18	50.86	.22
743	13.76	8.46	77.78	22.24	55.54	.12
134	12.42	7.93	79.65	18.31	61.34	.12
852	11.61	7.99	80.40	20.62	59.38	.15
910	15.56	7.02	77.42	16.28	61.14	.11
915	11.21	5.97	82.82	19.43	63.39	.09
934	14.06	5.46	80.48	10.93	69.55	.04
1942	12.36	8.72	78.92	13.52	64.44	.09
1943	8.41	6.28	85.31	....	....	....
1944	15.89	8.13	75.98	....	....	....
1945	12.91	5.71	81.36	....	69.98	.11
1946	15.23	8.52	76.25	....	....	.18
1947	13.29	7.50	79.21	....	....	....
948	14.43	5.26	70.31	....	....	....
Average	14.40	6.55	78.54	15.28	63.79	.097

TABLE 40.

Composition of Clay Precipitates—Per Cent.

Number	Loss on Ignition	Carbon	Hydrogen	Humus (c x 1.724)	Loss on Ignition Less Humus
1942	12.36	5.64	1.35	9.72	2.64
1943	8.41	2.11	.53	3.64	1.77
1944	15.89	6.54	1.39	11.25	4.64
1947	13.29	3.28	2.59	5.65	7.54
127	15.54	2.10	......	3.62	11.82
133	14.49	2.05	......	3.53	10.96
742	20.48	7.80	......	13.45	7.03
743	13.76	3.49	......	6.02	7.74
948	14.43	4.65	......	8.02	6.41
934	14.06	1.95	......	3.36	10.70
182	15.70	1.53	......	2.64	13.04
910	15.56	1.63	......	2.81	12.65
1946	15.23	4.61	......	7.95	7.28
332	18.34	4.52	......	7.79	10.55
1945	12.91	3.26	......	5.62	7.29
Average	14.61	3.68	1.46	6.34	8.38

TABLE 41.

Nitrogen in Clay Precipitate.

	Total	Nitrogen as Ammonia	Organic
133	.88	.65	.23
134	1.67	1.36	.31
182	.97	.87	.10
852	1.64	1.38	.28
910	2.40	2.35	.05
915	1.35	1.04	.31
101	1.42	1.22	.20
127	1.40	1.21	.19
332	1.27	1.11	.16
934	1.27	1.05	.22
324	1.64	1.50	.14

The clay precipitate also contains nitrogen. A portion of this is ammoniacal nitrogen, retained by the clay from the ammoniacal liquid and the ammonium salt used to precipitate it. A portion of the nitrogen is organic nitrogen. The percentage of organic nitrogen in the clay precipitate is greater than in the soil originally used.

The ammoniacal nitrogen in the clay would also contribute to the loss on ignition.

SUMMARY AND CONCLUSIONS.

1. The removal of the suspended clay is the most difficult part of the estimation of humus.

2. The A. O. A. C. method brings less clay into suspension than Snyder's method, and is more rapid with many soils.

3. A correction of the loss on ignition by subtracting 10 per cent of the ash as proposed by Peters and Averitt is better than no correction.

4. Filtration through unglazed porcelain removes the clay, but a portion of the organic matter may not pass through.

5. The clay may be precipitated by means of various salts, such as ammonium sulphate or chloride and sodium sulphate or chloride.

6. Electrolysis to remove clay will be studied further.

7. Evaporation and solution of the residue in ammonia as proposed by Mooers appears to be the most promising method for the estimation of humus.

8. Precipitation with acid, as is to be expected, removes only a portion of the dissolved organic matter. The average recovery is 64 per cent.

9. Extended washing with acid increases the quantity of ash in suspension but has little effect on the organic matter.

10. Strong ammonia extracts more organic matter than dilute ammonia. This is evidence that material goes into solution which is not "ammonium humate" but is merely organic matter soluble in ammonia, or converted by it into soluble compounds.

11. Organic matter added to the soil already contains ammonia-soluble material.

12. When no correction is made for the ammonia-soluble substances in the added material, ammonia-soluble humus is apparently formed in the decay of cottonseed meal, etc., but when correction is made for the added ammonia-soluble material the ammonia-soluble material is found to decrease.

13. The least loss of organic matter takes place with a soil containing water equal to 77 per cent of its saturation capacity.

14. "Humic acid" was prepared by two methods, with ammonia and with sodium hydroxide and sodium phosphate.

15. Humic acid shaken with salt solution according to Hopkins' method for soil acidity exhibits only about 12 per cent of its real acidity.

16. Precipitation of ammonium humates with various salts is discussed.

17. Magnesium salts do not precipitate some humates, and magnesium humate is much more easily soluble in water than calcium humate.

18. Humic acid is dissolved by caustic soda in presence of calcium carbonate.

19. Analyses of humic salts gave an equivalent of about 250 for the mixed humic acids.

20. Humic acid boiled with hydrochloric acid gave about 2 per cent reducing sugars.

21. The humus preparations contain easily diffusible material, when

dissolved in ammonia. After two or three diffusions, the "ammonium humate" diffuses at the nearly constant rate of about 1 to 2 per cent of the humate.

22. The quantity of phosphoric acid in the humus preparations by ammonia varies from 0.13 to 0.54 per cent, which is a small amount.

23. Our purified humic acids (by ammonia) contains 44-56 per cent carbon and 4.3 to 5.4 per cent nitrogen.

24. Humic acid extracted by phosphate contains a high amount of ash. The water and ash-free material contains 52 to 64 per cent carbon and 3.7 to 5.2 per cent nitrogen.

25. The humic acids from the California soils are not rich in nitrogen.

26. Humus soluble in alcohol contain higher percentages of carbon than that not soluble.

27. The clay precipitated by ammonium salts from the humus solution contains from 1.53 to 7.80 per cent carbon, equivalent to 2.64 to 13.45 per cent organic matter. The suspended clay may thus contain considerable percentages of organic carbon.

28. The loss on ignition, less the organic matter, varies from 1.77 to 13.04 per cent of the clay. The average is 8 per cent. This is the amount of the correction which should be made, if any is to be made.