

SOME CHEMICAL PROPERTIES ASSOCIATED WITH
AGGREGATION IN SOILS

by

THOMAS ALOYSIUS WELDON

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INTRODUCTION

The importance of organic matter in the soil has been recognized since the earliest soil investigations and an extensive literature has accumulated. Much of the early work directed toward the determination of the quality of organic matter in soils consisted in isolating various fractions that were soluble in alkali. These fractions have been called "humic acid," "ulmin," "hymalometanic acid," and various other chemical names but no definite chemical formula has been given for any one of them.

The importance of soil organic matter in the formation of soil aggregates has only recently received major attention (13).

Hide and Metzger (5) showed that the well aggregated fraction of a soil was higher in carbon and total nitrogen than was the poorly aggregated fraction. By proximate analysis of the poorly aggregated and the well aggregated fractions of various soils, it was hoped that differences in the quality of the organic matter might be found which might indicate why the well aggregated fraction is higher in carbon and total nitrogen than is the poorly aggregated fraction and thus help to determine some of the factors associated with aggregation in soils. Various conditions such as the type of material added to the soil, the soil organisms present, pH of the soil, aeration, and temperature and moisture relationships influence the rate of decomposition of organic matter added to the soil and probably

nature of the organic residues remaining in the soil. All of the above factors may influence aggregation either directly or indirectly.

REVIEW OF LITERATURE

A review of the literature revealed that before 1926 comparatively little fractionation of organic matter by methods similar to those now in use had been attempted. Since that time a number of investigations have been undertaken to determine whether soil organic matter from different sources is qualitatively different.

Waksman (18) found that the lignins and not the celluloses or hemicelluloses contributed to the formation of humus. He believed that humus formation was a result of the interaction of carbohydrates with amino acids or polypeptides.

Phillips, Weihe, and Smith (12) found rapid decomposition of the pentosans and the celluloses by soil organisms. Under proper conditions of aeration, temperature and moisture the soil organisms are also capable of decomposing lignin as found in plant materials.

Waksman and Stevens (21) proposed a method of organic matter fractionation which has been used in most of the recent work on fractionation of soil organic matter. The authors found that by their method 90 to 95 percent of all the constituents of the soil organic matter could be accounted for in groups of definite chemical complexes. They believed that the methods for measuring the abundance of "humus" or "humic acid"

based upon its extraction with alkalis could not be used for determining quantitatively the soil organic matter or even a definite fraction of it. The use of oxidizing agents such as permanganate solution or 6 percent hydrogen peroxide solution could not be used for determining the degree of decomposition. By the authors' method the soil organic matter was found to be composed largely of two groups of complexes: the lignin-like complexes or "soil-lignin," and the nitrogenous complexes or soil "proteins." In addition fats, waxes, and other ether or alcohol soluble substances as well as certain carbohydrates were found.

That the preservation of nitrogen in a sand medium depends mainly on the form in which nitrogen is added and the nature of the organic substances which serve as sources of humus was shown by Waksman and Hutchings (19). Carbohydrates, by supplying a readily available source of energy for the soil organisms, result in nitrogen being transformed into microbial cell substances. Lignin acts as a buffer for the absorption of ammonia and in the direct fixation of some of the protein.

In a later paper (20) the above authors found the chemical nature of organic matter in different soil types to be different.

Maddock (9) working on the decomposition of organic materials in both natural and synthetic soils found that a pH of 5.5 gave best results with NaNO_3 as a nitrogen source. Fungi were active in the beginning stages of decomposition, but later when the reaction changed toward neutrality the bacteria

and actinomycetes became active. Dyal, Smith and Allison (2) likewise found that the rate of decomposition was more rapid and complete in soils whose pH values ranged between 5.94 and 7.04 than in soils with pH values between 3.71 to 4.59. The detrimental effect of acidity was more pronounced on natal grass than on crotalaria.

Lockett (7) in his study on the decomposition of clover and rye plants at different growth stages found that young plants contain a larger proportion of fats and other ether and alcohol soluble substances, carbohydrates, proteins and soluble ash than do older plants. Toward maturity the content of the hemicelluloses, celluloses and lignins increased progressively. The higher the concentration of the water-soluble constituents, especially the carbohydrate and nitrogen fractions, the more rapid the decomposition occurred. In a later paper (8) the author found that the lignin and protein complexes account for most of the residual organic matter in young plants while hemicellulose, cellulose and lignin account for most of the organic matter remaining in the compost of mature plants.

Sokoloff (15) found that sodium, either adsorbed or present in the soil solution, tends to promote depletion of the organic reserves of the soil. The release of nitrates and of total nitrogen, as well as the soluble and volatile forms of carbon, is stimulated, often to a great extent, by the sodium salts and depressed by the corresponding calcium salts, regardless of aeration. The carbon-nitrogen ratio of the undissolved organic matter is lower in the sodium treated than in the calcium

treated aerated soils.

In the Lake States Region, Wild, Buran and Galloway (23) found that the nutrient content and base exchange properties of organic matter are influenced not only by the type of organic material deposited but that the underlying mineral soil also exerts an important effect in the development of organic remains.

Studying Kansas soils Metzger (10) found that nitrogen and carbon losses were similar on a 16-year rotation, a three-year rotation, and continuous wheat. The 16-year rotation lost nitrogen more rapidly than the other two cropping systems, but the three-year rotation led in loss of carbon. Alfalfa grown continuously increased the soil's supply of nitrogen and carbon at the rate of 0.71 percent and 0.43 percent per year respectively.

Wheeting (22) studying the changes in organic matter in western Washington soils as a result of cropping found that there was a 28 percent increase in the quantity of organic matter under cropping and likewise the quality of the organic matter was also improved probably because of changes from the woody forest type of organic matter to the more easily broken down cereal type.

The greatest increase occurred where good management practices were followed and under such treatments western Washington cropped soils have maintained a superiority with regard to organic matter over virgin soils for at least a 45-year cropping period.

In Utah Stevens (17), working with an arid soil under irrigation, found the soils to be somewhat high in the ether and

alcohol-soluble fractions, the "protein" and "lignin-humus" fractions, were also high, but the carbohydrate fraction was exceedingly low.

Shewan (16) working with north-east Scottish soils, and using a modification of the Waksman and Stevens method for fractionating the organic matter, divided the soils studied into a raw humus and a mull type according to the manner in which the various fractions have been decomposed. The hemi-cellulose was fairly similar in all the samples studied, the cellulose varied considerably, lignin was fairly constant, but the protein was usually higher in the deciduous litter.

Studying the nature of organic matter in western Washington prairie soils as influenced by differences in rainfall, Fowler and Wheeting (3) found that the nitrogenous complexes together with the "lignin-humus" complex constitute the major portion of the soil organic matter. The carbon-nitrogen ratio and total nitrogen content in the organic matter were found to vary in relation to the mean annual rainfall; the carbon-nitrogen ratio was high under high rainfall and narrowed under low rainfall, while the nitrogen content of the organic matter was low under high rainfall and increased with decreasing amounts of rainfall. The water soluble materials were least abundant in the soils developed under heavy rainfall.

Gillam (4) found the chemical nature of humic acids from a grassland soil of the Great Plains, a Minnesota muck, and a Michigan forest soil to be very similar in all physical and chemical properties. These humic acids were readily acetylated

and methylated; all three possessed hydroxyl groups which could be esterified or methylated but which do not undergo base exchange; potentiometric curves indicate the presence of a carboxyl group; and the non-nitrogenous fraction of humic acid consists of a slightly modified lignin complex. The differences noted between the humic acids were few and consisted of slight variations in base exchange capacities and titration curves.

From some of the preceding articles it is seen that the quantity and quality of the soil organic matter differs in some respects depending on the location, treatment, parent materials, pH, and methods of fractionation. However, indications from Gillam's (4) type of fractionation show the organic matter to be similar under different conditions.

Leighty and Shorey(6) found from a study of a large number of samples taken from various parts of the United States that the carbon-nitrogen ratio may vary within the wide limits of 3.5 to 35.2 but that the average is close to 10.

MATERIALS AND METHODS

In this study both a poorly aggregated fraction and a well aggregated fraction were isolated from each of 12 different samples, including eight different soil types. Surface soil only, taken to a depth of approximately 8 to 10 inches, was used in all cases. Three of the samples were taken on virgin areas only, one sample from an idle field previously cultivated, and the other eight samples included both a virgin and a

Table 1. Pertinent data with regard to the soils used in the experiment.

Sample	Soil type	Origin	Cultural Condition	Location
1	Geary silt loam	Loessial	Virgin	0.2 miles north of the west corner of the Agronomy Farm.
2	Geary silt loam	Loessial	Cultivated	20 yards due east of sample 1.
3	Wabash silty clay loam	Alluvial	Virgin	0.9 miles north of the east corner of the Agronomy Farm.
4	Wabash silty clay loam	Alluvial	Cultivated	15 yards due west of sample 3
5	Idana silty clay loam	Residual	Virgin	5 miles northwest of Manhattan
6	Idana silty clay loam	Residual	Cultivated	50 yards due east of sample 5.
*7	"Slick spot"	Loessial	Virgin	150 yards due north of sample 1.
8	"Slick spot"	Loessial	Cultivated	75 yards southeast of sample 7.
9	Summit silt loam	Residual	Virgin	3½ miles east, 3 miles south, and ¼ mile east of Dover.
10	Carrington loam	Glacial	Virgin	6 miles north of Topeka on Highway 75.
11	Oswego clay loam	Residual	Virgin	3 3/4 miles south of Willard.
12	Holdredge silt loam	Loessial	Cultivated	10 miles east of Russell.

*Sample was taken from a narrow fence row and may not be a typical "slick spot" surface soil although the sample was taken above subsoil showing definite "slick spot" characteristics.

cultivated sample from each of four separate soil types. The soils used in this study are arranged in Table 1 according to sample number, type, origin, cultural condition, and location.

The surface soil was removed to a depth of approximately 8 to 10 inches and allowed to air dry on top of a laboratory desk. The clods were gently broken when first brought into the laboratory, but the material was not sieved as a previous trial using the same soil and sieving through a one-fourth inch sieve while still moist resulted in a very low yield of the well aggregated material and a correspondingly high yield of the poorly aggregated material. Fifty grams of the air dry soil were placed in beakers, distilled water poured down the side of each beaker until the soil was covered and allowed to stand three hours. At the end of this time the wet soil was washed into a tall cylinder nearly full of distilled water and then the cylinder was filled. The volume of the cylinder was 1645 cc and its diameter was three and one-fourth inches. To exclude all air bubbles during mixing a tube was fitted in the center to allow a small amount of liquid to escape when the cork was placed in the cylinder. This tube was later closed for mixing. The cylinder was inverted 10 times, placed in a vertical position and allowed to stand 30 seconds. At the end of this time the upper 1000 cc was drawn off rapidly through a one-half inch siphon and considered as the poorly aggregated fraction. The well aggregated material was obtained by washing the remaining portion through a 24-mesh screen and using only material that would not pass through. By this method only those aggregates

stable in water remained on top of the sieve. Probably some of the water stable aggregates were broken in the process, however. No attempt was made to determine the amount of soil needed for the isolation. The soils varied widely in the amount of sample that had to be used in order to obtain the necessary amounts of the two fractions. Two of the soils offered a special problem. In the Summit silt loam, sample 9, some difficulty was encountered in obtaining a poorly aggregated fraction while in both the cultivated and the virgin "slick spots," it was impossible to obtain enough of the water stable aggregated material to work with. In this case aggregated material not passing through a 40-mesh screen was used rather than that which would not pass through one of 24-mesh. These were the only exceptions made in the isolation process.

The material containing the poorly aggregated fraction was siphoned into a five gallon bottle and after allowing to settle for 24 hours the material still in suspension was precipitated with aluminum sulfate and the supernatant liquid which appeared perfectly clear was drained off and discarded. The remaining material was dried slowly on a steam bath. No difficulty was experienced in drying the aggregated fraction as the water was easily decanted. This material was also slowly dried on a steam bath.

The dried material was ground to pass through a 100-mesh sieve, moisture determinations made, and the samples placed in soil cans for future use.

Methods Used for Fractionating the Organic Matter

The method of proximate analysis used by Shewan (16) was adopted as a basis for the experimental work. Some modification in the procedure was necessary in order to be able to use the method with this particular problem.

The procedure adopted for the analysis of the organic matter in the two fractions was as follows:

Nitrogen, Carbon, Acidity and Moisture Determinations. On separate portions of the original soil the percent moisture was determined by drying at 105°C for 18-24 hours; the percent nitrogen was obtained by digesting according to the Gunning-Hibbard procedure, distilling into a four percent boric acid solution and titrating with N/7 H_2SO_4 using a mixture of methyl-red and brom-cresol-green as indicator; the acidity was determined by using the glass electrode; the organic carbon was determined by using the Schollenberger method as outlined by Allison (1).

Ether-Alcohol Extraction. Fifty grams of air-dry soil were extracted for 30 hours in a Soxhlet apparatus with ethyl-ether, the residue after the evaporation of the ether was dried in an oven at 100°C for one-half hour, placed in a desiccator and weighed after cooling. The soil after the ether extraction was transferred to a beaker and again allowed to air dry. Forty grams of the air-dry, ether extracted material was placed in a 500 cc Erlenmeyer flask, 150 cc of 95 percent ethyl alcohol

added and boiled on a sand bath under a reflux condenser for two hours. The hot alcohol was filtered through a Buchner funnel, using a Whatman No. 42 filter paper. The soil was thoroughly washed with hot alcohol. The residue, after the evaporation of the alcohol was dried at 100°C for one-half hour, cooled in a desiccator and weighed.

Hemicellulose, Total Nitrogen, Amide Nitrogen. The ether-alcohol extracted material was transferred to a beaker and allowed to air dry. Twenty-five grams of this material was placed in a 500 cc Erlenmeyer flask, 350 cc of a two percent HCl solution was added and boiled under a reflux condenser for five hours. After cooling, the solution was filtered and made up to a volume of one liter. On a 200 cc aliquot reducing sugars were determined according to the Schaffer and Hartman method (14). These determinations were run in duplicate and calculated for the total original sample. The iron and aluminum precipitated from 200 cc of the solution was ignited in a muffle furnace, cooled, weighed, and these weights calculated for the total original sample. On a 500 cc portion total nitrogen was determined as previously outlined after evaporating most of the water. On a 250 cc portion amide nitrogen was determined by neutralizing the acid with milk of lime, adding five grams of "heavy" magnesium oxide, distilling the ammonia into a four percent boric acid solution and titrating with $N/7 \text{ H}_2\text{SO}_4$ in the presence of brom-cresol-green and methyl red.

Cellulose. The soil after hydrolysis with HCl was allowed to air dry, 20 grams were placed in a 500 cc Erlenmeyer flask,

25 cc of 80 percent H_2SO_4 added and allowed to stand for two and one-half hours at room temperature after which time 325 cc of distilled water was added and the mixture boiled under a reflux condenser for five hours. On cooling the solution was filtered and made up to a volume of one liter. Reducing sugars, total nitrogen, amide nitrogen and the weights of iron and aluminum were determined as before.

Lignin-Humus and Protein Nitrogenous Complexes. The soil from the H_2SO_4 extraction was air dried and total nitrogen and organic carbon determined by the methods previously outlined. The lignin-humus complexes were calculated for the two fractions using the following formula as outlined by Waksman and Stevens (21):

$$\text{Percent of "lignin-humus" complex in soil} = \frac{a \times 100}{A} - \frac{b \times 100}{S}, \text{ where } a = \text{the carbon content in}$$

the sulfuric acid residue, calculated on the basis of the total original sample of soil, A = the total carbon content of the sample of soil, b = protein content in the sulfuric acid residue, obtained by multiplying the nitrogen content of the residue by 6.25, S = total organic matter in the soil sample, as calculated from the organic carbon of the soil.

The protein content was determined by multiplying the total nitrogen of the soil by 6.25; the quantity thus obtained is divided by the total organic matter of the soil and multiplied by 100 to give the percentage of protein in the soil organic matter. All the results are stated on the basis of total organic matter, i.e. carbon percentage multiplied by 1.724.

Throughout the course of the experiment small samples had to be used and any experimental error was thus magnified. This

is especially true in determining amide nitrogen and as only a small sample was available no duplicates could be run.

Organic Carbon, pH, and Carbon-Nitrogen Ratio at the
Beginning of the Experiment and at the
End of the Experiment

From Table 2 it is apparent that the amount of organic carbon is distinctly higher in the well aggregated than in the poorly aggregated fractions. In only one soil, sample 10 was the organic carbon lower in the well aggregated than in the poorly aggregated fraction, and in this sample the well aggregated fraction had observable amounts of subsoil material scattered throughout. These results are in agreement with those reported by Hide and Metzger (5). The carbon content was higher in both the well aggregated and the poorly aggregated fraction of the virgin samples than in the cultivated samples in three of the four soils studied. Expressing the carbon content of the poorly aggregated fraction as percentage of the well aggregated fraction at the beginning of the experiment the average figure was 68.5 while this same percentage figure at the end of the experiment was 68.0. Likewise, expressing the carbon content of the well aggregated material after the various extractions as percentage of the well aggregated material at the beginning of the experiment the average figure was 65.2 while the same percentage figure on the poorly aggregated material was 64.7.

The amounts of carbon extracted by the various treatments from samples from the normal cultivated fields were similar in both the well aggregated and poorly aggregated fractions. This

Table 2. Organic carbon content of the well aggregated and poorly aggregated fractions at the beginning and at the end of the study.

Sample	Original fraction			After treatments		
	Organic carbon		P_1/A_1 percent	Organic carbon		P_2/A_2 percent
	A_1^*	P_1^*		A_2	P_2	
1	2.39	1.97	82.4	1.62	1.19	73.5
2	1.92	1.45	75.5	1.24	0.95	76.6
3	3.18	2.00	62.9	2.29	1.54	67.2
4	3.43	3.03	88.3	2.50	2.16	86.4
5	3.84	2.02	52.6	2.10	1.44	68.6
6	1.31	1.04	79.4	0.73	0.59	80.8
7	4.72	3.00	63.5	3.60	2.09	58.1
8	3.62	0.44	12.1	2.50	0.24	9.6
9	2.61	2.51	96.1	1.75	1.60	91.4
10	1.44	1.53	106.1	0.71	0.77	108.4
11	2.79	2.23	79.9	1.57	1.23	78.3
12	1.49	1.18	79.2	0.78	0.72	92.3
Ave.	2.73	1.87	68.5	1.78	1.21	68.0

*For convenience in preparation of the tables the symbol "A" will be used to refer to the well aggregated fraction and will be subnumbered for each time it appears in the tables while "P" will refer to the poorly aggregated fraction and will be similarly subnumbered.

indicates similarity of organic matter. The amounts extracted from the two fractions of the cultivated "slick spot" and from all virgin samples, however, differ considerable, indicating differences in the nature of the organic matter. An interesting feature of the data for the percentage carbon was that the virgin "slick spot," sample 7, was higher in percentage of carbon in the well aggregated fraction than any of the other soils while the poorly aggregated fraction lacked only 0.03 percent of being equal to the highest figure in this fraction. The well aggregated fraction of the cultivated "slick spot," sample 8, likewise was quite high in percentage of carbon while the poorly aggregated fraction from this same sample had by far the lowest percentage of carbon of any of the soils studied. Expressing the carbon content of the poorly aggregated fraction as a percentage of that in the well aggregated fraction the virgin sample was 63.5 percent while the cultivated sample was only 12.1 percent.

Ether and Alcohol Extracts

It is apparent from Table 3 that the amount of ether and alcohol soluble material is not distinctly different in the well aggregated and poorly aggregated fractions although a number of trends are apparent. The portion of the organic matter soluble in ether was lower in the well aggregated than in the poorly aggregated fraction of the cultivated soil in four of the five cases. This trend is not apparent in the virgin samples. In the well aggregated fractions the ether

Table 3. The ether-alcohol soluble materials expressed as percentage of total organic matter.*

Sample	Ether			Alcohol		
	A_1^{**}	P_1^{**}	P_1/A_1 percent	A_2	P_2	P_2/A_2 percent
1	4.44	1.76	39.6	3.39	5.32	156.9
2	2.50	3.08	123.2	5.31	4.11	77.4
3	1.44	2.02	133.3	2.04	4.61	225.9
4	1.31	0.92	70.2	3.80	3.78	99.5
5	2.10	2.42	115.2	3.39	4.20	123.9
6	1.19	3.26	273.9	6.07	5.68	93.5
7	1.06	0.70	66.0	2.26	2.40	106.2
8	1.38	2.37	171.7	3.30	24.68	747.9
9	0.87	1.48	170.1	8.35	2.77	33.1
10	2.04	1.71	83.8	14.73	6.52	37.4
11	1.55	1.73	111.6	3.77	4.30	114.1
12	1.36	1.50	110.2	4.17	5.04	120.8

*Total organic matter was obtained by multiplying percent carbon by 1.724.

**Refer to footnote in Table 2.

soluble separate is higher in the virgin samples than in the cultivated samples in three of the four cases while in the poorly aggregated fractions the cultivated samples tend to be higher in ether soluble material. This would indicate a tendency for the aggregates containing ether soluble material to be broken down as a result of cultivation.

The alcohol soluble separate was lower in the well aggregated fraction than in the poorly aggregated fraction in five of the seven virgin samples. In the cultivated samples three of the five samples were higher in the well aggregated than in the poorly aggregated fraction. Also the well aggregated fractions from the virgin samples were lower in alcohol soluble material than were the corresponding well aggregated materials from the cultivated samples in the four cases studied. No trend is apparent in the poorly aggregated fraction. Evidently cultivation tends to increase the amount of alcohol extractible material in the well aggregated fraction without exerting any definite influence on the poorly aggregated fraction. The alcohol soluble material in sample 8 was very much different from that in the other cultivated samples studied. There was approximately seven and one-half times as much of the organic matter soluble in alcohol in the poorly aggregated fraction as there was in the well aggregated fraction. Of the total organic matter in this poorly aggregated sample, 24.68 percent was alcohol soluble while in the other soils studied the percentage ranged from 2.4 percent to 6.52. There was approximately ten and one-thirds times as much alcohol soluble organic matter

in the poorly aggregated fraction of the cultivated "slick spot" as there was in the virgin "slick spot." Evidently the alcohol soluble fraction was not conducive to aggregate formation in this cultivated "slick spot" as this was the sample in which difficulty was encountered in securing an aggregated sample. Although a sample from only one cultivated "slick spot" site was used it appears that cultivation exerts a much more marked influence on the alcohol soluble material in this soil than it does in any of the other soils studied.

Hemicellulose

It is apparent from Table 4 that the amount of hemicellulose and cellulose is not distinctly different in the well aggregated and the poorly aggregated fractions although certain trends can be observed. The portion of the organic matter broken down by the HCl treatment and representing the hemicellulose fraction did not vary widely in most cases between the well aggregated and the poorly aggregated fractions for the same soil. The average amount of hemicellulose material present in both the well aggregated and the poorly aggregated fractions was 9.59 percent and 8.49 percent, respectively, although in five of the twelve cases the trend was reversed. Samples 7 and 8 are outstanding in having very small amounts of hemicellulose in the well aggregated fraction and none in the poorly aggregated fraction.

Cellulose

In Table 4 it may be seen that the results secured for the cellulose fraction are variable with no definite trends indicated. Sample 8 was outstanding in having an exceptionally high percentage of cellulose in the poorly aggregated fraction, almost one-fourth of the organic matter being cellulose. This high cellulose content was not evident in the similar fraction of the virgin "slick spot" sample. Samples 3 and 4, both good bottom soils, had very low percentages of cellulose in the well aggregated and the poorly aggregated fractions.

Lignin-Humus

From Table 4 it is apparent that the amount of lignin-humus is not distinctly different in the two fractions although a number of trends can be observed. In both the well aggregated and the poorly aggregated fractions the percentage of lignin-humus is higher in the well aggregated virgin sample than in the cultivated sample in three of the four cases studied although the exceptions do not occur on the same pairs of samples. The well aggregated fraction in the cultivated soils was higher in lignin-humus than the poorly aggregated fraction in three of the four samples, but no trend was evident in the virgin samples. Wide fluctuations were found in the well aggregated fraction ranging from a low of 33.22 percent in the glacial soil, sample 10, to a high of 64.69 percent in the virgin "slick spot," sample 7. In the poorly aggregated fraction, sample 10, with

Table 4. Carbohydrate separates from the well aggregated and poorly aggregated fractions expressed as percentage of the original organic matter.

Sample	Hemicellulose			Cellulose			Lignin		
	A ₁ *	P ₁ *	P ₁ /A ₁ percent	A ₂	P ₂	P ₂ /A ₂ percent	A ₃	P ₃	P ₃ /A ₃ percent
1	18.40	12.73	69.2	6.71	7.72	115.0	57.71	49.14	85.1
2	12.40	14.66	118.2	3.97	4.63	116.6	52.97	51.24	96.7
3	12.27	5.71	46.5	1.61	2.10	130.6	60.76	65.40	107.6
4	11.88	12.55	105.6	1.50	0.98	65.3	62.75	49.18	78.9
5	7.82	10.48	134.0	8.02	13.07	162.9	46.22	61.18	132.1
6	12.13	7.04	58.0	10.12	2.40	23.7	37.75	53.57	141.9
7	1.43	0.00	0.0	4.43	5.08	114.6	64.68	57.69	89.1
8	1.67	0.00	0.0	3.10	24.50	790.3	56.62	37.11	65.5
9	9.39	10.54	112.2	14.33	4.48	31.2	52.92	57.50	108.6
10	10.55	9.79	92.8	7.18	1.11	15.5	33.22	36.32	109.0
11	10.71	10.33	86.4	3.16	1.94	61.4	48.49	37.41	77.1
12	6.42	8.08	125.8	7.62	7.40	97.1	36.36	51.99	142.9
Ave.	9.59	8.49	89.7	5.98	6.29	143.7	50.87	50.64	102.9

* Refer to footnote in Table 2.

a percentage of 36.33 was again low while sample 3 with 65.4 percent was the highest. Since the average percentages for both fractions show very little difference it is evident that the lignin-humus complexes are similar in the two fractions from the standpoint of both average amount and extreme range of values. The variations in the lignin-humus complexes were much greater than those found by Waksman and Stevens (21), Shewan (16), Fowler and Wheeting (3), and Stevens (17). Although carbon was higher in the well aggregated fraction than in the poorly aggregated fraction no single carbon separate showed this trend.

Total Nitrogen

The data in Table 5 show that the total nitrogen is distinctly higher in the well aggregated fraction than in the poorly aggregated fraction in 10 of the 12 samples. Sample 10 which was only 0.0055 percent lower in nitrogen in the well aggregated fraction had considerable quartz in this fraction which was coarser than the screen through which the poorly aggregated material had passed. This quartz was discarded where possible. Although a rubber pestle was used in grinding the sample there was a possibility that some of the quartz was included in the ground material. The nitrogen content was higher in both the well aggregated and the poorly aggregated fractions of the virgin samples than in the cultivated samples in three of the four soils. The soil higher in nitrogen content in the cultivated sample likewise was higher in

carbon. Expressing the nitrogen content of the poorly aggregated fraction as a percentage of the nitrogen content of the well aggregated fraction at the beginning of the experiment, the average figure was 69.1 percent while the same percentage figure at the end of the experiment was 61.4, or 88.9 percent as much as the poorly aggregated material was extracted as of the well aggregated material. Expressing the nitrogen content of the well aggregated material after the various extractions as a percentage of the nitrogen content of the well aggregated material at the beginning of the experiment, the average figure was 44.5 percent while the same percentage figure on the poorly aggregated material was 39.6 percent. This would indicate that on an average the nitrogenous materials in the poorly aggregated fractions are somewhat more stable than those found in the well aggregated fractions, and apparently there may be a qualitative difference in the nitrogen extractible materials in the two fractions. An interesting fact observed in the data was that the well aggregated fraction from the virgin "slick spot," sample 7, was higher in total nitrogen than any of the other soils studied while the poorly aggregated fraction ranked second in total nitrogen content among the poorly aggregated fractions. The well aggregated fraction from the cultivated "slick spot," sample 8, ranked second in total nitrogen but the poorly aggregated fraction had by far the lowest amount of total nitrogen. Expressing the poorly aggregated fraction as a percentage of the well aggregated fraction the virgin sample was 63.5 percent

while the cultivated sample was 12.3 percent. These percentages are practically identical with the figures obtained for the carbon and show the exceptionally low content of carbon and nitrogen in a poorly aggregated sample of a cultivated "slick spot."

Nitrogen Extracted by Treatment with a Two Percent HCl Solution

From Table 5 it is observed that an average of 44.3 percent of the nitrogen compounds in the well aggregated fractions was broken down by the HCl treatment while an average of 38.5 percent of the nitrogen compounds in the poorly aggregated fractions was broken down by the same treatment. Thus 86.9 percent as much of the poorly aggregated material as of the well aggregated material was extracted. At the beginning of the experiment the poorly aggregated fractions contained 68.9 percent as much nitrogen as the well aggregated samples while after the HCl extraction it contained only 60 percent as much nitrogen, again showing that a lower proportion of the poorly aggregated material was attacked by the HCl treatment. The conclusion that usually between one-third and one-half of the total nitrogen is released by treatment with HCl seems justified. These figures are in agreement with Shewan's (16) work on Scottish Soils. While the actual amount of amide nitrogen in the HCl hydrolyzate was slightly higher in the well aggregated fraction, it makes up only 30.1 percent of the total material in this fraction while 44.1 percent of

the nitrogen compounds of the poorly aggregated HCl hydrolyzate was in the amide form. This indicates that an appreciably higher proportion of amide nitrogen is present in the poorly aggregated fraction of the HCl hydrolyzate than in the well aggregated fraction. In the well aggregated fraction 13.6 percent of the soil nitrogen was in the amide form while 19.5 percent of the soil nitrogen was in this form in the poorly aggregated fraction. These figures are higher than Shewan's (16) who found that between five and seven percent of the nitrogen was in the amide form.

Nitrogen Extracted by Treatment with an 80 Percent H_2SO_4 Solution

An average of 22.9 percent of the original nitrogenous material was extracted from the well aggregated fraction with 80 percent H_2SO_4 as shown in Table 5 while an average of 18.8 percent of the nitrogen content of the poorly aggregated material was liberated by the same treatment. This means that only 82.1 as much of the poorly aggregated material as of the well aggregated material was extracted. This lower amount of the nitrogenous compounds of the poorly aggregated material extracted by the H_2SO_4 is further emphasized by the fact that the nitrogen content of the poorly aggregated fraction at the beginning of the experiment was 68.9 percent of the amount present in the well aggregated material while the nitrogen content of the poorly aggregated H_2SO_4 hydrolyzate was 56.9 percent of the well aggregated hydrolyzate. From the data presented it appears that about one-fifth of the total

nitrogen was removed by the H_2SO_4 treatment which is also in agreement with Shewan (16). Thirty-three and two-thirds percent of the nitrogen in H_2SO_4 hydrolyzate in the well aggregated fraction was in the amide form while 40.4 percent of the poorly aggregated material was in this form. This also indicates that more of the nitrogen in the poorly aggregated fraction is in the amide form than was true in the HCl hydrolyzate. In both the well aggregated and the poorly aggregated fractions 7.6 percent of the total nitrogenous materials was extracted in the amide form by H_2SO_4 .

Expressing the amide nitrogen in the H_2SO_4 hydrolyzate as percentage of that of the amide nitrogen in the HCl hydrolyzate in both fractions, the well aggregated sample shows a value of 56.5 percent while the same value for the poorly aggregated sample is 44.9 percent. There was, therefore, approximately one-half as much amide nitrogen in the H_2SO_4 hydrolyzate as there was in the HCl hydrolyzate. The total amide nitrogen in both the HCl and the H_2SO_4 extractions in the well aggregated fractions was 21.2 percent of the soil nitrogen while 27.2 percent of the soil nitrogen in the poorly aggregated fraction was in this form.

Protein Nitrogen

Table 5 shows no distinct differences in the protein nitrogen complexes although certain trends are indicated. In seven of the twelve cases the well aggregated fraction was slightly lower in protein nitrogen than the poorly aggregated

Table 5. Total nitrogen of the fractions and nitrogen data following the various treatments expressed as percent by weight of the original fractions.

Sample	Beginning			End					HCl hydrolyzate					Amide nitrogen from HCl hydrolyzate				
	A ₁ *	P ₁ *	P ₁ /A ₁ percent	A ₂	P ₂	P ₂ /A ₂ percent	A ₂ /A ₁ percent	P ₂ /P ₁ percent	A ₃	P ₃	P ₃ /A ₃ percent	A ₃ /A ₁ percent	P ₃ /P ₁ percent	A ₄	P ₄	P ₄ /A ₄ percent	A ₄ /A ₁ percent	P ₄ /P ₁ percent
1	.1848	.1662	89.9	.0667	.0614	92.0	36.0	36.9	.0910	.0748	82.1	49.2	46.0	.0130	.0292	224.6	7.03	17.5
2	.1635	.1244	76.0	.0769	.0573	74.5	47.0	46.0	.0622	.0520	83.6	38.0	41.8	.0229	.0227	99.1	14.00	18.4
3	.2219	.1298	58.4	.0992	.0587	59.1	44.7	45.2	.0930	.0805	86.5	41.9	62.0	.0064	---	---	2.80	----
4	.2242	.1988	88.6	.0964	.0790	81.9	43.0	42.9	.1003	.0631	62.9	44.7	31.7	.0388	.0323	83.2	17.30	16.2
5	.2775	.1516	54.6	.0902	.0604	66.9	33.1	39.8	.0979	.0700	71.5	35.2	46.1	.0652	.0241	36.9	23.40	15.8
6	.1256	.0918	73.0	.0653	.0091	13.9	52.0	9.9	.0428	.0218	50.9	34.0	23.7	.0185	.0162	87.5	14.70	17.6
7	.3079	.1957	63.5	.1519	.0996	65.5	49.3	50.8	.1232	.0837	67.9	40.0	42.7	---	---	----	---	----
8	.3018	.0372	12.3	.1248	.0212	16.9	41.3	56.9	.1145	.0191	16.6	37.9	51.3	---	---	----	---	----
9	.1790	.2020	112.8	.1022	.0435	42.5	57.1	21.5	.0863	---	----	48.2	----	---	.0278	----	---	13.7
10	.1230	.1285	104.4	.0641	.0594	92.6	52.1	46.2	---	---	----	----	----	.0182	.0147	80.7	14.70	11.4
11	.1860	.1540	82.7	.0601	.0517	86.0	32.3	33.5	.1048	.0488	46.5	56.3	31.6	.0360	.0244	67.7	19.30	15.8
12	.1020	.0734	71.9	.0660	.0558	84.5	64.7	76.0	.0579	.0700	120.8	56.7	95.3	.0231	.0196	84.8	22.60	26.7
Ave.	.1998	.1378	74.0	.0886	.0547	64.7	45.6	42.1	.0885	.0531	60.0	44.2	38.5	.0269	.0234	96.8	13.60	19.5

*Refer to footnote in Table 2.

(Right hand margin continued)

Table 5. (concl.)

Sample	H ₂ SO ₄ hydrolyzate					Amide nitrogen from H ₂ SO ₄ hydrolyzate					Protein nitrogen	
	A ₅ *	P ₅ *	P ₅ /A ₅ percent	A ₅ /A ₁ percent	P ₅ /P ₁ percent	A ₆	P ₆	P ₆ /A ₆ percent	A ₆ /A ₁ percent	P ₆ /P ₁ percent	A ₇	P ₇
1	.0384	.0383	99.7	20.7	23.0	.0101	.0121	119.8	5.4	7.2	27.90	30.46
2	.0372	.0304	81.7	22.7	24.4	.0121	.0081	66.9	7.4	6.5	30.69	31.00
3	.0535	.0232	43.3	24.1	17.8	.0181	.0141	77.9	8.1	10.8	25.17	23.45
4	.0519	.0456	87.8	23.1	22.9	.0163	.0121	74.6	7.2	6.0	23.59	23.67
5	.0000	.0420	----	----	----	.0245	.0161	65.7	8.8	10.6	26.08	27.08
6	.0326	.0220	67.5	25.9	27.7	.0106	.0101	95.2	8.4	11.0	34.58	31.32
7	.0486	.0349	71.8	15.7	17.8	.0148	.0082	55.4	4.8	4.1	23.53	23.52
8	.0545	.0120	22.0	18.0	32.2	.0147	---	----	4.8	----	30.08	30.60
9	.1097	.0112	10.2	20.0	5.5	.0267	.0163	61.0	14.9	8.0	24.75	29.02
10	.0113	.0061	53.9	9.2	4.7	.0124	.0122	98.3	10.0	9.4	30.87	30.33
11	.0391	.0269	68.7	21.0	17.4	---	.0021	----	----	1.3	24.07	24.93
12	.0261	.0189	72.4	15.5	25.7	.0068	.0042	61.7	6.6	5.7	24.71	22.48
Ave.	.0457	.0259	61.7	19.6	18.7	.0152	.0105	77.7	7.6	7.6	27.17	27.32

*Refer to footnote in Table 2.

fraction and in one remaining soil both fractions were the same. The variations between the different soils were not marked. In the well aggregated fraction sample 7 was low with 23.53 percent protein nitrogen while sample 6 was high with 34.58 percent. In the poorly aggregated fraction sample 12 was low with 22.48 percent while sample 6 was high with 31.32 percent. Sample 6 had the highest percentage of protein nitrogen in both fractions. Since the average percentages for the two fractions show very little difference it is evident that the protein nitrogen complexes are similar in the two fractions from the standpoint of both average amounts and extreme range of values.

The average percentages of protein nitrogen complexes are lower than reported by Waksman and Stevens (21) slightly higher than those reported by Fowler and Wheeting (3) and much lower than those reported by Stevens (17).

Carbon Nitrogen Ratio

The carbon-nitrogen ratios as presented in Table 6 varied from 10.4 to 15.3 in the well aggregated fractions and from 11.3 to 16.1 in the poorly aggregated fractions, but the average was 13.4 for both fractions. This figure is somewhat higher than the old accepted value of 10 but is within the range occurring in soils as found by Leighty and Shorey (6). The average carbon-nitrogen ratio is similar to that found by Hide and Metzger (5) although they found the carbon-nitrogen ratio to be slightly wider for the well aggregated

fraction than for the poorly aggregated fraction.

Recovery of Organic Matter

The percentage recovery of organic matter was obtained by adding the percentage of the total organic matter extracted by each of the treatments. As shown in Table 6 the percentage recovery was over 100 in six of the well aggregated samples, and seven of the poorly aggregated samples. There is no definite trend on those samples running over 100 percent. There are two places in the procedure used in which there might be a probable explanation for the high percentage recovery in some of the soils. The factor used in the Schollenberger method as modified by Allison (1) for organic carbon determination may have been too low in some of the soils giving a low percentage of organic matter which would account for a high percentage recovery of organic matter. A more likely cause for the wide variation in percentage recovery of organic matter is the conversion factor 1.724 used to convert percent carbon to percent organic matter. Schewan (16) states that the figure 1.724 appears too low to use on Scottish soils. He found a range of 1.79 to 2.09 and suggested using the figure 1.92. Had a higher conversion factor been used the percentage recovery would have been less than 100. If it be true that the factor is variable then the accepted one of 724 would be misleading if applied to all soils.

pH Values

The data presented in Table 6 show that, while none of the original samples had values above pH 7.0, only two of the samples were below pH 6.0. The well aggregated fractions had slightly higher pH values in eight of the 10 comparisons. In the cultivated "slick spot" the well aggregated fraction was slightly on the alkaline side while the poorly aggregated fraction had a very low pH value. As this represents only one site, it was not possible to know if this condition generally exists in all cultivated "slick spots." Likewise no apparent reason can be offered for the low pH value in the poorly aggregated fraction of sample 9.

R_2O_3 Precipitated from the HCl and H_2SO_4 Hydrolyzate

Table 6 shows that the well aggregated material in every case yielded more R_2O_3 in the HCl hydrolyzate than the poorly aggregated fraction. Expressing the R_2O_3 as a percentage of the organic matter the well aggregated fraction was distinctly higher than the poorly aggregated fraction. Evidently the R_2O_3 found in the well aggregated fractions is more easily removed by weak acid treatment than the R_2O_3 found in the poorly aggregated fractions.

Metzger (11) in studying the fixation of phosphorus in relation to the iron and aluminum of the soil concluded that the organic matter exerts a reducing effect on the iron in the soil and thus the iron is more easily extracted by treatment

Table 6. Carbon-nitrogen ratios, pH values, percentage recovery of organic matter, and the percentage of R_2O_3 found in the HCl and H_2SO_4 hydrolyzates.

Sample	C/N ratio		pH			Recovery of organic matter		R ₂ O ₃ in acid hydrolyzate (percent of original soil sample)									
								HCl hydrolyzate					H ₂ SO ₄ hydrolyzate				
	A ₁ *	P ₁ *	Field sample	A ₂	P ₂	A ₃	P ₃	A ₄	P ₄	P ₄ /A ₄	A ₄ /O.M.**	P ₄ /O.M.	A ₅	P ₅	P ₅ /A ₅	A ₅ /O.M.	P ₅ /O.M.
						percent	percent			percent	percent	percent			percent	percent	percent
1	12.9	11.9	6.3	6.2	6.7	118.3	107.1	3.18	3.14	98.7	76.6	92.0	2.04	1.84	90.1	49.2	53.8
2	11.7	11.7	5.8	6.1	5.9	107.8	108.7	3.34	3.00	89.8	100.2	86.6	3.02	1.90	62.9	90.6	75.6
3	14.3	15.4	---	6.2	6.3	103.3	103.3	3.98	3.20	80.4	72.2	92.4	1.08	0.94	87.0	19.6	27.2
4	15.3	15.2	---	---	6.0	104.8	91.1	4.56	3.86	84.6	76.6	73.4	1.48	0.38	25.6	25.0	7.2
5	13.8	13.3	6.1	5.9	---	93.6	118.4	4.72	1.42	30.0	70.8	40.4	3.90	1.32	45.5	43.6	37.6
6	10.4	11.3	6.3	6.5	6.2	101.5	103.3	6.06	2.14	35.3	266.8	118.8	3.36	2.26	67.2	148.0	125.6
7	15.3	15.3	6.8	6.4	5.9	97.4	89.4	4.32	1.76	40.7	52.8	33.8	1.74	1.20	68.9	21.2	23.2
8	12.0	11.8	6.9	7.3	4.2	96.1	119.3	9.74	0.64	6.5	155.2	84.2	4.06	0.58	14.2	64.6	76.2
9	14.3	12.4	6.1	6.2	4.3	101.6	105.8	5.48	2.12	38.6	121.2	48.6	5.12	3.92	76.5	113.2	90.0
10	11.7	11.9	5.6	5.6	4.4	98.6	85.8	4.56	1.56	34.2	183.0	58.8	5.46	2.68	49.0	219.2	101.2
11	15.0	14.5	6.7	6.8	5.7	91.7	80.6	4.18	1.54	36.8	86.4	99.8	2.00	1.02	51.0	41.4	26.4
12	14.6	16.1	6.9	6.3	6.3	80.6	96.5	4.80	1.96	38.7	186.0	91.0	0.82	4.24	517.0	31.6	208.0
Ave.	13.5	13.4						4.90	2.18	44.4	120.6	71.6	2.84	1.86	67.3	72.2	71.0

*Refer to footnote in Table 2.

**O. M. refers to organic matter.

with dilute acid than is the iron in a soil containing less organic matter. As the poorly aggregated fractions were lower in organic matter than the well aggregated fraction, this probably accounts for the smaller amounts of R_2O_3 extracted from this fraction.

In the H_2SO_4 hydrolyzate the well aggregated fraction was richer in R_2O_3 than the poorly aggregated fraction in 11 of the 12 samples. There was no trend in the amount of R_2O_3 extracted as compared to the organic matter present as was found to be the case in the HCl hydrolyzate. There was more R_2O_3 extracted by the HCl treatment than by the H_2SO_4 treatment in the well aggregated fraction in 11 of the 12 samples while in the poorly aggregated fraction, 9 of the 12 were higher in the HCl extraction. There was a greater difference in the amount of R_2O_3 extracted from the two fractions by the HCl treatment than by the H_2SO_4 treatment.

SUMMARY AND CONCLUSIONS

1. Twelve soils selected from a number of sites in Kansas and representing various types, origins, and treatments were selected for this study. From each soil a well aggregated fraction and a poorly aggregated fraction were separated and analyzed according to methods of proximate analysis as outlined by Waksman and Stevens (21) and as modified by Shewan (16).

2. The ether soluble material accounted for only a small percentage of the total organic matter, and while the alcohol

soluble fraction amounted to about three times that of the ether soluble fraction, it still made up only a small percentage of the organic matter.

3. The carbon and total nitrogen contents were higher in the well aggregated fractions than in the poorly aggregated.

4. About one-third of the total nitrogen was released in the HCl treatment and one-fifth in the H_2SO_4 treatment while only about one-eleventh of the carbonaceous material was released from the HCl treatment and one-sixteenth from the H_2SO_4 treatment. There was about one-sixth of the total nitrogen in the HCl hydrolyzate in the amide form and about one-twelfth from the H_2SO_4 hydrolyzate in this form. The poorly aggregated fractions were considerably higher in amide nitrogen than the well aggregated fractions. The lignin-humus and the protein nitrogen complexes were similar in the two fractions from the standpoint of average amounts and extreme range of values.

5. The percentage recovery of organic matter obtained by adding the percentage of the total organic matter extracted by each of the treatments was rather widely variable in the two fractions. The amount of R_2O_3 was higher in the well aggregated fraction in the HCl hydrolyzate in all cases and the same was true in 11 of the 12 cases for the H_2SO_4 treatment. There appears to be a higher percentage of R_2O_3 extracted from the well aggregated HCl hydrolyzate than from the poorly aggregated fraction on the basis of total organic matter, but this is not true for the H_2SO_4 hydrolyzate.

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