THE ORGANIC MATTER OF THE SOIL: II. A STUDY OF CARBON AND NITROGEN IN SEVENTEEN SUCCESSIVE EXTRACTS; WITH SOME OBSERVATIONS ON THE NATURE OF THE BLACK PIGMENT OF THE SOIL¹

By

ROSS AIKEN GORTNER, *Agricultural Experiment Station, University of Minnesota*

INTRODUCTION

It has already been shown in the first paper of this series (2) that:

1. Humus, the "matière noire" of Grandeau (3), does not consist entirely of a black compound or compounds but contains a large proportion of almost colorless substances, the presence of which is normally masked by the black color.

2. Extracts can be obtained from unchanged vegetable materials which are very similar in solubility, color, etc., to "humus" extracts prepared from soils.

3. A 4 per cent solution of sodium hydroxide does not extract either the same substances or the same quantity of substances as does a 4 per cent solution of ammonium hydroxide.

4. All of the mineral soils tested contain a "soil pigment" which is soluble in ammonia and very dilute sodium hydroxide to form intensely black solutions, but which is insoluble in sodium hydroxide of 4 per cent concentration.

5. This soil pigment contains a relatively small proportion of the soil nitrogen and it was suggested that it probably had but little importance, *per se*, in the problem of soil fertility.

In view of the above findings it seemed advisable to attempt to isolate this soil pigment in a pure form. Such an attempt is recorded in this paper. It is to be regretted that the final preparations contain such a high percentage of ash as probably to invalidate the ultimate analyses, but it is impossible to carry the investigation further at the present time, and the results to date seem worthy of record.

¹ The work reported in this paper was carried out while the author, now Associate Agricultural Bio-Chemist, was Associate Soils Chemist.

Received for publication September 13, 1916.

EXPERIMENTAL

The soil selected for this investigation was from a bulk sample of the first 6 inches of the Fargo silt loam type (4), the air-dry soil containing 12.70 per cent of moisture. The soil contained 9.90 per cent of carbonate CO_2 , 10.04 per cent of organic carbon and 0.908 per cent of nitrogen, all calculated on the basis of oven-dry soil.

In the above mentioned paper (2) the writer has shown that while the soil pigment is insoluble in 4 per cent sodium hydroxide, it is readily soluble in the dilute alkaline solution formed when distilled water is added to the soil residue remaining after siphoning off the 4 per cent sodium hydroxide solution. An attempt was made to utilize this phenomenon for the purpose of separating the soil pigment. One kg. of the air-dry soil, ground to pass a 1-mm. sieve, was leached in 50 gm. lots on filters with 1 per cent HCl until the filtrate gave no test for calcium, and the excess of acid removed by washing with water. The leached soil was then removed to 2½-liter bottles, the soil residue from 100 gm. of the original air-dry soil being placed in each bottle and 2 liters of 4 per cent NaOH added to each. The bottles were shaken several times each day for 3 days and then the soil was allowed to settle until the supernatant liquid appeared to be free from clay, 3 to 4 days usually being allowed. The supernatant liquid was siphoned off as completely as possible without disturbing the soil, measured and united in a large container, 16,900 c.c. being removed. This constituted "NaOH Solution A." Fresh 4 per cent NaOH solution was added to the soil residue until 2 liters of NaOH were again contained in each bottle and the process repeated until there had been 9 successive extractions with 4 per cent NaOH.

Carbon and nitrogen determinations were made of each solution, the carbon being estimated by wet combustion in the apparatus figured in the preceding paper (2), corrections being made for the CO_2 of carbonates in the sodium hydroxide solutions.¹

The color of the solutions rapidly decreased in intensity, the last two extractions being practically colorless.

Following the nine extractions with sodium hydroxide the contents of one of the bottles was made slightly acid with sulfuric acid, the precipitated soil washed by decantation until practically free from sulfates, then poured upon a filter and washed free of sulfates, removed from the filter, dried, weighed and powdered to pass a 0.5-mm. sieve. From the

¹ It was impossible to secure accurate organic carbons on the large volume (45,350 c.c.) of HCl extract and washings, so that the organic carbon in this portion was determined by difference.

Ten gm. of the air-dry soil were leached on an alundum filter with 1 per cent HCl until free from calcium, and washed free of chlorides with distilled water. The soil was then washed from the filter into an evaporator, dried and weighed. The 10 gm. of original air-dry soil, containing 0.8764 gm. of organic carbon, gave 6.4452 gm. of dry leached soil containing (1) 13.12 per cent, and (2) 13.10 per cent, or an average of 13.11 per cent, equivalent to 0.8450 gm. of organic carbon. The HCl leachings therefore dissolved 0.0314 gm. of organic carbon.

original 87.30 gm. of soil (dry) there was obtained a residue weighing 41.5 gm. or 47.53 per cent of the original soil weight. This residual soil contained 6.27 per cent of organic carbon and 0.346 per cent of nitrogen.

A "humus" determination was made on this residual soil by shaking 5 gm. of the sample with 750 c.c. of 4 per cent NH_4OH for one week, flocculating the clay with ammonium carbonate, filtering and evaporating 250-c.c. portions to dryness in tared silica dishes. Duplicate determinations gave 8.38 and 8.45 per cent of the residual soil as ash-free humus of an average of 8.42 per cent. The humus-ash percentages were 1.76 and 1.80 per cent.

The "humus" solution was jet black and on drying the product scaled away from the dish in flakes where the deposit had any appreciable thickness. The dissolved material apparently consisted of the black soil pigment combined with ammonia, inasmuch as the product after drying at 105°C . for 12 hours was still readily soluble in water. Detmer (1) has observed that a soluble "ammonium humate" can be formed by dissolving "humic acid" in ammonia and evaporating the mixture to dryness. The properties of his "salt" resemble very closely those of the writer's product.

To the remaining nine soil residues, distilled water was added to 2 liters volume, the mixture thoroughly shaken and 5 gm. of crystallized sodium sulfate added to each bottle to flocculate the clay. After several shakings the soil was allowed to settle and the black supernatant liquid siphoned off, this constituting Pigment Solution 1. Additional 0.25 per cent sodium sulfate solution was added to the residue and the process repeated until six pigment solutions had been removed.¹

Pigment Preparation I was obtained from the combined Pigment Solutions 1, 2 and 3, and the remaining solutions 4, 5 and 6 furnished Pigment Preparation II (see below).

The residual soil from the pigment extractions was acidified slightly with sulfuric acid, washed by decantation to the absence of acid reaction, poured upon filters and washed to the absence of sulfates, dried, weighed and powdered to pass a 0.5-mm. sieve. There was thus obtained a light gray residue weighing 330.5 gm. as compared with an original weight of 785.7 gm. (900 gm. air-dry) and containing 3.59 per cent of organic carbon and 0.211 per cent of nitrogen.

Inasmuch as it was thought probable that there was still some of the "soil pigment" remaining in the residue, "humus" was determined on the resulting residue. Two 15-gm. portions were leached with 1 per cent HCl and washed free of chlorides as in the usual humus method. This treatment was resorted to in order to get the soil in as near as possible the same physical state as it would have been had it not been previously dried and ground. The 15 gm. of soil were then added to 750-c.c. portions

¹ Fifteen c.c. of 20 per cent NaOH were added to each 2000 c.c. of solution used for the extraction of Pigment Solutions 5 and 6.

of 4 per cent NH_4OH in separate bottles and shaken for one week in a shaking machine so arranged that the bottles were rotated sidewise "wheel fashion" so that the soil was continually falling through the solution. At the end of a week the bottles were removed, the clay precipitated in a portion of the solution with K_2SO_4 and carbon determined on the jet black solution as in the writer's earlier directions (2). The remaining portion was used for "humus" determinations after flocculating the clay with ammonium carbonate as in Rather's (5) method. The residual soil was found to contain 1.54 per cent of humus (ash-free), 0.74 per cent of "humus ash," and 0.95 per cent of "humus carbon" indicating 62.25 per cent of carbon in the humus. This carbon percentage agrees fairly well with the carbon content of Pigment Preparation I, *i. e.*, 61.33 per cent (see later).

The summarized analytical data are shown in Tables I and II. Figure 1 is a graphic representation of the data of Table II.

The analytical results obtained on this series are conclusive proof of the accuracy of my wet combustion apparatus for the determination of organic carbon. The increase of 1.534 gm. of carbon amounts to an error of 1.76 per cent of the total carbon. Inasmuch as carbon was determined on 50-c.c. portions of the solution, there would have been enough solution to make 5,012 carbon determinations, not considering the 45,350 c.c. of the HCl extract; a gain of 1.534 gm. of carbon, therefore, amounts to an average gain of only 0.0003 gm. of carbon per determination. A nitrogen gain of 0.1590 gm. N is similarly an average gain of only 0.00013 gm. N per determination, not counting errors in the nitrogen content of the original soil nor of the final soil residue.

Pigment Preparation 1. Pigment Solutions 1, 2 and 3 were united and acidified with sulfuric acid. A jet black flocculent precipitate separated leaving the supernatant liquid absolutely colorless. The precipitate was washed by decantation with distilled water until the supernatant liquid began to take on a brown tint showing that the pigment was beginning to form a colloidal solution. Sufficient sodium hydroxide solution was then added to form a 0.2 per cent solution. The precipitated pigment was dissolved to a jet black solution, which was only faintly alkaline to litmus. This solution was poured upon a large filter and the filtrate diluted to 7 liters in volume. One liter of sodium hydroxide solution containing 320 gm. of sodium hydroxide was then added, thus making the solution to contain 4 per cent of sodium hydroxide. The black pigment was precipitated in this concentration of alkali, leaving a supernatant liquid which appeared very dark when in large bulk but which was relatively light colored when in small volume. This supernatant liquid was analyzed for organic carbon and nitrogen and gave a ratio of C/N of 15.77. This is decidedly lower than the C/N ratio of the original solution, 21.0, and would indicate that the substances dissolved here were perhaps normally soluble in 4 per cent NaOH. It is

to be noted that this ratio of 15.77 logically follows the C/N ratio of NaOH solution 1.

The supernatant liquid was removed from the pigment as completely as possible by centrifuging, and the solid mass of pigment added to 8 liters of distilled water in which it readily dissolved to form a jet black solution. This solution was filtered by gravity through double filters and

TABLE I
SUMMARIZED ANALYTICAL DATA ON THE SOILS AND SOLUTIONS¹

	Volume of Solution Removed from 1 kg. Soil ²	Organic Carbon			Nitrogen		
		Per cent I	Per cent II	Per cent Av.	Per cent I	Per cent II	Per cent Av.
1% HCl Extract	c.c.						
45,350	45,350	0.353	0.368	0.360	0.025	0.023	0.024
NaOH Solution—							
A.....	16,900	3.353	3.323	3.338	0.363	0.364	0.363
B.....	17,100	1.126	1.145	1.136	0.138	0.138	0.138
C.....	17,300	0.640	0.626	0.633	0.080	0.080	0.080
D.....	17,600	0.442	0.424	0.433	0.053	0.053	0.053
E.....	17,700	0.298	0.299	0.298	0.033	0.033	0.033
F.....	17,300	0.252	0.252	0.252	0.026	0.027	0.026
G.....	17,500	0.173	0.180	0.177	0.018	0.017	0.018
H.....	17,000	0.143	0.151	0.147	0.012	0.012	0.012
I.....	15,900	0.101	0.108	0.104	0.010	0.009	0.009
Residual Soil from NaOH Solution I..		3.007	2.953	*2.980	0.163	0.165	*0.164
Pigment Solution—							
1.....	15,890	0.168	0.160	0.164	0.008	0.009	0.009
2.....	18,890	0.475	0.474	0.475	0.020	0.021	0.020
3.....	17,112	0.595	0.591	0.593	0.029	0.029	0.029
4.....	14,445	0.292	0.290	0.291	0.015	0.015	0.015
5.....	14,110	0.164	0.183	0.173	0.006	0.006	0.006
6.....	15,890	0.104	0.102	0.103	0.006	0.003	0.004
Residual Soil from Pigment Solution 6		1.521	1.503	1.512	0.088	0.089	0.088
Total				10.189			0.927
Original Soil		10.076	10.020	10.040	0.904	0.913	0.908

¹ All percentages are based on the weight of the original soil (dry at 105°).

² The volume for the pigment solutions has been increased 1/9 to correspond to 1 kg. of soil instead of 900 gm. actually extracted (see text).

* Not included in the additions for "Total."

the filtrate acidified slightly with sulfuric acid. The precipitate was washed by decantation until it began to form a colloidal solution, then poured on a filter and washed until practically free from sulfates. The large mass of gelatinous precipitate, probably approximating 800 c.c. in bulk, was dried on a water bath. In drying it cracked into small pieces which resembled anthracite in color and hardness when perfectly dry. The yield was approximately 16 gm.

The crude pigment was powdered, extracted on an alundum filter with hot water to remove traces of sulfates, then extracted in a Soxhlet apparatus with alcohol. The extract rapidly darkened and finally became deep orange in color. On evaporating off some of the alcohol and cooling, a very considerable quantity of colorless crystals separated. These crystals have not as yet been identified.

The extracted pigment was dried at 105° C. and analyzed for carbon, hydrogen, nitrogen and ash in the usual manner. The product as prepared forms a jet black amorphous powder, insoluble in alcohol, ether, chloroform or any of the usual organic solvents, insoluble in dilute acids,

TABLE II
SHOWING THE PERCENTAGE OF THE TOTAL CARBON AND NITROGEN IN THE
DIFFERENT SOILS AND SOIL EXTRACTS

	Gm. C.	Per cent Total C.	Gm. N.	Per cent Total N.	C/N Ratio
1000 gm. Original Soil	87.640		7.9300		11.05
1% HCl Extract	3.137	3.58	0.2192	2.76	14.32
NaOH Solution—					
A.....	29.139	33.25	3.1730	40.01	9.18
B.....	9.910	11.31	1.2034	15.17	8.24
C.....	5.524	6.30	0.6972	8.79	7.92
D.....	3.782	4.32	0.4611	5.81	8.20
E.....	2.607	2.98	0.2867	3.61	9.09
F.....	2.199	2.51	0.2318	2.92	9.49
G.....	1.756	2.00	0.1505	1.90	11.67
H.....	1.278	1.46	0.1054	1.33	12.13
I.....	0.919	1.05	0.0668	0.84	13.76
Residual Soil from NaOH Solu. I	¹ 26.020	¹ 29.69	¹ 1.4350	¹ 18.09	18.12
Pigment Solution—					
1.....	1.421	1.62	0.0747	0.94	19.03
2.....	4.152	4.74	0.1813	2.29	22.90
3.....	5.181	5.91	0.2498	3.15	21.06
4.....	2.537	2.89	0.1271	1.60	19.96
5.....	1.531	1.75	0.0522	0.66	29.34
6.....	0.901	1.03	0.0365	0.29	24.66
Residual Soil from Pig't Solu. 6	13.200	15.06	0.7730	9.75	17.08
Total	89.174	101.76	8.0900	101.82	

¹ Not included in the additions for "Total."

soluble in concentrated sulfuric acid to a black solution, dissolving slightly in hot concentrated nitric acid to a red-brown color, soluble in ammonia or very dilute solutions of the fixed alkalis to intensely black solutions, from which the pigment can be again precipitated by solutions of the heavy metals or by acidification.

When alkaline solutions of the pigment are dialyzed in collodion bags, the excess of alkali escapes but no pigment passes through. In this manner neutral solutions of the pigment can be easily prepared, which yield a black water-soluble residue when evaporated to dryness. These probably correspond in nature to the ammonium compound obtained by evaporating an ammoniacal solution of the pigment. Whether we are

Fig. 1.—A graphic representation of the data in Table II.

dealing with a true salt in this instance can be decided only by further experimentation on purer material. The following analytical data were obtained on the sample:

Ash determinations indicated 37.50, 37.74 and 37.16 per cent or an average of 37.47 per cent of ash.

Organic carbon (by CuO in oxygen) determinations gave 61.28, 61.59 and 61.12 per cent C, or an average of 61.33 per cent of carbon calculated to an ash-free basis.

Hydrogen determinations gave 4.35, 4.25 and 4.37 per cent hydrogen, or an average of 4.36 per cent calculated ash-free.

Nitrogen determinations gave 2.79 and 2.82 per cent N, or an average of 2.81 per cent calculated ash-free.

Sulfur was not tested for because of the high ash content and also because sulfuric acid had been used to precipitate the pigment.

These analyses therefore would indicate an approximate content of:

Carbon	61.3 per cent
Hydrogen	4.3 per cent
Nitrogen	2.8 per cent
Oxygen	31.6 per cent

It is useless to compare these analyses with the analyses of "humic acids," etc. in the literature until this product can be prepared in a purer state. It is of interest to note, however, that the analyses approximate those of Detmer's (1) "humic acid," which contained 60.74 per cent C, 4.48 per cent H, and 35.78 per cent O. Detmer's product contained smaller amounts of nitrogen, ranging from 0.79 per cent to 0.18 per cent, and he was inclined to the belief that his "humic acid" was nitrogen-free. The physical properties of his material are almost identical with those of the soil pigment used by the writer. He does not state, however, whether or not his "humic acid" is insoluble in 4 per cent NaOH and soluble in the very dilute solutions; this would appear to be the distinguishing feature of the soil pigment.

The high ash content consists presumably of colloidal clay which was not removed by the preliminary treatment. This clay undoubtedly holds a certain amount of combined moisture that was not driven off by the drying at 105° C. so that it appears probable that the hydrogen content as determined is too high. Some diminution of ash could be secured by dissolving the pigment in ammonia and precipitating the clay with ammonium carbonate. This was what was actually done in the humus determination on the residual soil from NaOH solution 1. This soil residue was found to contain 8.42 per cent of humus with 1.78 per cent of humus ash, or an ash content of only 17.45 per cent in the materials not precipitated by the ammonium carbonate. The ammonia treatment would be far more rapid than the sodium hydroxide process but was not

utilized because of the danger of introducing ammonia into the compound and thus increasing the nitrogen content. Inasmuch as the writer's analyses now show the approximate nitrogen content, it is extremely desirable that some one should attempt to isolate the pigment by the ammonia process. Grandeau (3) found that when an ammoniacal solution of humus containing 53 per cent of ash was dialyzed against distilled water for 36 hours, the resulting residue in the dialyzer had an ash content of only 8 per cent. Perhaps this method could be employed in freeing the soil pigment from ash.

Pigment Preparation 2. This sample of pigment, weighing approximately 7 gm. was prepared from Pigment Solutions 4, 5 and 6, following much the procedure as was used for Pigment Preparation 1, with the exceptions that the solution of the pigment in dilute alkali was not precipitated by adding NaOH up to 4 per cent concentration and that hydrochloric acid was used for acidification instead of sulfuric. The final extractions were the same for both preparations. The following analytical data were obtained on the sample:

Ash determinations indicated 50.77 and 51.57 per cent, or an average of 51.17 per cent of ash.

Organic carbon (by CuO in oxygen) determinations gave 55.82 and 56.23 per cent C, or 56.03 per cent of organic carbon calculated to an ash-free basis.

Hydrogen determinations gave 5.89 and 6.36 per cent, or 6.13 per cent calculated ash-free.

Nitrogen determinations gave 2.94 and 3.02 per cent N, or an average of 2.98 per cent calculated to an ash-free basis.

These analyses would indicate an approximate content of

Carbon	56.03 per cent
Hydrogen	6.13 per cent
Nitrogen	2.98 per cent
Oxygen	34.86 per cent

It will be noted that this preparation contains appreciably more hydrogen and less carbon than Preparation 1. This is exactly what would be expected from a sample having a higher ash content, if the ash consisted of clay. Apparently the clay has retained enough water of constitution to vitiate entirely the hydrogen analysis and to lower the carbon percentage appreciably.

SUMMARY

A sample of soil of the Fargo silt loam type was leached with 1 per cent hydrochloric acid and then extracted 9 consecutive times with fresh portions of a 4 per cent sodium hydroxide solution. A sample of the residual soil was removed at this point and analyzed, while the remain-

ing portions were further extracted for 6 consecutive times with very dilute (approximately 0.15 per cent concentration) sodium hydroxide solution. Following these extractions the remaining soil residue was dried and analyzed. The following conclusions are evident from the analytical data:

1. The writer's earlier observation that 4 per cent sodium hydroxide solution does not dissolve the black soil pigment is confirmed.
2. The soil pigment is soluble in very dilute sodium hydroxide solutions but precipitates from solution on the addition of sodium hydroxide in sufficient amount to make a 4 per cent solution.
3. The soil pigment is also precipitated from solution by salts of the heavy metals and by acidification; is not dialyzable and forms a stable water-soluble compound with ammonia when an ammoniacal solution is evaporated to dryness.
4. Two attempts were made to prepare the soil pigment in pure form but the resulting products contained such a high content of ash (37.47 per cent and 51.17 per cent, respectively) as probably to render the ultimate analysis unreliable. The analysis of the product with the least ash content gave C., 61.3 per cent; H., 4.3 per cent; N., 2.8 per cent; O., 31.6 per cent; all calculated to an ash-free basis.
5. The first six extractions with NaOH removed relatively more nitrogen than carbon from the soil, but the remaining three NaOH extractions as well as the six pigment solutions contained relatively more carbon than nitrogen. The final soil residue had a ratio of C/N very much higher than that of the original soil.

LITERATURE CITED

- (1) DETMER, W.
1871. Die natürlichen Humuskörper des Bodens und ihre landwirthschaftliche Bedeutung. *In* Landw. Vers. Stat., Bd. 14, p. 248. Reprinted *in* Jahresber. Agr. Chem., 1870-72, p. 68-72.
- (2) GORTNER, R. A.
1916. The organic matter of the soil: I. Some data on humus, humus-carbon and humus-nitrogen. *In* Soil Sci., v. 2, p. 395-442.
- (3) GRANDEAU, LOUIS.
1872. Recherches sur le rôle des matières organiques du sol dans les phénomènes de la nutrition des végétaux. *In* Compt. Rend. Acad. Sci. [Paris], t. 74, p. 988-991.
- (4) MARBUT, C. F., BENNETT, H. H., LAPHAM, J. E., and LAPHAM, M. H.
1913. Soils of the United States. U. S. Dept. Agr. Bur. Soils Bul. 96. 791 p., 13 fig., 2 maps.
- (5) RATHER, J. B.
1911. An improved method for the estimation of humus. *In* Jour. Indus. Engin. Chem., v. 3, p. 660-662.