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SOLUBLE NONPROTEIN NITROGEN OF SOIL

By R. S. POTTER, *Assistant Chief in Soil Chemistry*, and R. S. SNYDER, *Assistant in Soil Chemistry, Iowa State College Experiment Station*

INTRODUCTION

Dilute alkali dissolves a larger proportion of the organic material of soil than any of the other relatively mild reagents. A still larger percentage is extracted from soil previously treated with 1 per cent of hydrochloric acid (HCl), and this latter reagent dissolves but little of the organic material. The term "humates" is fast disappearing from current scientific literature, yet one often reads that the reason the preliminary washing with acid renders the organic matter more soluble in the alkali is that the calcium of the calcium humates is dissolved out, making the free humic acids soluble in the alkali. To say that the proteins are rendered more soluble by the removal of the calcium and the heavy metals would explain the solubility just as well and would be more correct scientifically.

As pointed out by Lipman (4, p. 251), much of the organic nitrogen of the soil must be protein in nature. The chief sources of the nitrogen are crop residues, manures, and bacterial cells, and in these much of the nitrogen is in the form of protein. Investigations carried out in this laboratory (5) have shown that soils contain a large quantity of the so-called humin compounds. These have a great tendency to be adsorbed by such compounds as magnesium oxid and calcium hydroxid, and therefore removal of calcium from the soil by acid would tend to make these more soluble.

Upon the acidification of the alkali extract a precipitate is obtained which has been called humic acid. This term also is no longer taken seriously. It would seem that the rational explanation of this precipitate would be simply that it was made up of proteins thrown down, as salts of the precipitant, as salts of organic acids, such as nucleic acid (7), or resinous acids (6), both of the latter substances having been isolated from the acid precipitate. It would also contain, no doubt, some free organic acids.

In analyses of the solution obtained by the prolonged boiling of soils with strong acids and of the hydrolyzed humic acids by the Van Slyke method (8), it was found that the results for the humic acids did not differ markedly from the results for the organic matter of the soils as a whole from which they were derived. Since that time it has occurred to

the writers that this would hold for the material precipitated by acid from the alkali extract, but perhaps this would not be true of the organic nitrogen compounds remaining in solution. It has been pointed out by Shorey (7) that many organic compounds have been isolated from the alkali extract of soil, which, though relatively quite soluble in water, can not be detected in or isolated from the water extract of soils. Therefore it has seemed that information might be obtained relative to the degree of decomposition of the organic matter in the soil by determining the proportion of nitrogenous compounds left in solution after the precipitation of the proteins by a suitable reagent was completed. It was with these problems in mind that the preliminary investigation was carried out.

EXPERIMENTAL METHOD

The general procedure followed was to determine the nitrogen in the alkali extract of soil with and without added material and the determination of nitrogen in the filtrate from the precipitate of the proteins in the alkali extract of soil with and without added material. The recent critical examination of a few protein precipitants by Greenwald (3) led us to use trichloracetic acid as our precipitant.

The detailed procedure was as follows: Soil, ground to pass a sieve of 100 meshes to the inch, was extracted with 1 per cent of hydrochloric acid until no calcium was found in the wash water. After air drying, 100 gm. were placed in an 800 c. c. bottle and 500 c. c. of a 1.5 per cent solution of sodium hydroxid added. After shaking the mixture for 2 hours it was centrifuged for 5 minutes in a bowl centrifuge having a speed of 18,000 revolutions per minute. Two 25 c. c. portions of the clear but deeply colored extract were analyzed for total nitrogen by the Gunning-Arnold method. Two 25 c. c. portions were neutralized with sulphuric acid, sufficient trichloracetic acid in solution added to give a 2.5 per cent solution of the acid and a total volume of exactly 30 c. c. After centrifuging, 10 c. c. portions were taken from each tube and analyzed for nitrogen by the Bock and Benedict (1) modification of the Folin and Denis method (2). This was called the soluble nonprotein nitrogen.

The same procedure was used where material was added to the soil. In the case of guanin, hypoxanthin, and glucosamin,[1] weighed portions of the compounds were added to the soil, which was then shaken with the alkali. The hydrolyzed casein was prepared as directed by Greenwald (3), which consisted, in brief, of boiling the casein for 40 hours with hydrochloric acid, the removal of the acid under diminished pressure, neutralization with sodium hydroxid (NaOH), and filtration. After mixing the filtrate with animal charcoal it was again filtered and final filtration carried out after crystallization of the tyrosin. For all the remaining materials solutions were prepared, sometimes with the aid of a little *N*/10 acid or *N*/10 alkali. Suitable amounts of the solutions were

[1] We wish to express our thanks to Prof. P. A. Kober for the samples of the hypoxanthin and guanin, and to Dr. A. W. Dox for the sample of glucosamin.

added to the soils and then sufficient alkali added to make 500 c. c. of a 1.5 per cent solution. In all cases, with the above-noted exceptions, the purest commercially available compounds were used, but analyses for nitrogen were run on the solid material when it was used, and when solutions were employed aliquots were analyzed. These determinations were also made by the micro method. It should be mentioned here that 6 minutes was found to be quite an inadequate digestion period for some of the compounds. It is believed that in some cases when apparently more than 100 per cent of the added substance was extracted from soil, faulty analysis of the substance was the cause. Insufficiency of material precluded repeating tests with many of the materials.

The soil used for all these tests was a silt loam containing 0.30 per cent of nitrogen. Samples A and B, as shown in Table I, differ only in that they were not taken from the field at the same time.

TABLE I.—*Analyses of 5-gm. portions of soil for alkali-soluble and soluble nonprotein nitrogen*

Soil sample.	Substance added.	Nitrogen added.	Nitrogen in the alkali extract.	Nitrogen of the added substance recovered in the alkali extract.		Soluble nonprotein nitrogen.	Soluble nonprotein nitrogen recovered.	
		Mgm.	*Mgm.*	*Mgm.*	*Per ct.*	*Mgm.*	*Mgm.*	*Per ct.*
A	Nothing	.....	5.93	.....	.....	1.29	.....	.....
	Hydrolyzed casein	2.09	7.99	2.06	98.4	3.33	2.04	97.5
	Amino benzoic acid	2.14	8.08	2.15	100.4	3.41	2.12	99.0
	Glutamic acid	2.27	8.26	2.33	102.6	3.48	2.19	96.5
	Hippuric acid	2.42	8.40	2.47	102.0	3.73	2.44	100.8
	Glutamic acid imid	2.36	8.36	2.43	103.0	3.59	2.30	97.5
	Succinimid	2.53	8.45	2.52	99.5	3.86	2.57	101.5
	Guanidin sulphate	1.97	6.725	1.975	40.4	1.84	0.55	28.0
	Urea	2.48	8.41	2.48	100.0	3.66	2.37	95.6
	Uric acid	2.42	8.34	2.41	99.6	3.78	2.49	103.0
	Caffein	2.32	6.93	1.00	43.1	2.21	.92	39.7
	Theobromin	1.96	7.90	1.97	100.5	3.27	1.98	101.0
	Guanin	2.49	8.45	2.52	101.2	2.325	1.035	41.6
	Hypoxanthin	2.52	8.34	2.42	96.0	3.42	2.13	84.6
	Skatol	1.26	7.19	1.26	100.0	1.80	.51	41.2
	Nucleic acid	2.00	7.92	1.99	99.5	1.67	.38	19.0
	Cadaverin	2.00	7.92	1.99	99.5	2.45	1.16	58.0
	Amygdalin	2.48	8.41	2.48	100.0	3.42	2.13	85.7
	Peptone (Witte)	2.98	8.76	2.83	94.9	2.12	.83	33.5
	Casein	2.42	7.42	1.49	61.6	1.29	0	0
	Edestin	2.43	6.58	.65	26.8	1.29	0	0
	Egg albumin	2.01	7.56	1.63	81.3	1.29	0	0
	Glucosamin	1.98	8.08	2.15	108.5	3.36	2.07	104.5
B	Nothing	.....	6.51	.....	.....	1.25	.....	.....
	Asparagin	2.11	8.62	2.11	100.0	3.24	1.99	94.2
	Acetanilid	2.14	8.66	2.15	100.5	3.24	1.99	93.0
	Benzamid	2.17	8.69	2.18	100.5	3.27	2.02	93.2
	Creatinin	2.07	8.06	1.55	74.8	2.71	1.46	70.5

It is not thought that all the compounds used are actually present in soil. The substances were chosen rather to represent classes of compounds which conceivably might be in soils. Guanin, hypoxanthin, nucleic acid, peptone, and creatinin have been isolated from soil. It is

realized that the list is very incomplete. As opportunity to make or to purchase more compounds presents itself, the investigation will be considerably extended. From the data presented, it is observed that quite varying proportions of the pure proteins, which in reality are soluble in dilute alkali, are extracted. This seems to be a confirmation of the contention that the alkali extract as a whole does not represent a definite class of nitrogenous compounds. Of the simpler compounds, it is seen that the more acid and more closely neutral compounds are completely extracted and remain in the soluble nonprotein portion. An exception to this is found in the case of nucleic acid. This is to be expected from its tendency to combine with protein compounds to give insoluble nucleins. The action of the purin compounds is interesting. In general the more basic the compound the less the quantity recovered.

CONCLUSIONS

(1) If the results with the pure proteins be considered, it is probable that the alkali extract as a whole contains no definite group of compounds.

(2) From the results obtained by the precipitation of the alkali extract with trichloracetic acid it would seem that the soluble nonprotein fraction may contain most of the simpler nitrogenous compounds, and therefore its determination would give an index of the degree of decomposition of the organic matter in the soil.

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