LABORATORY 9

SOIL ORGANIC MATTER AND EXTRACTION OF HUMUS

I Objectives

Extract humus and demonstrate cation exchange and flocculation / dispersion behavior.

II Introduction

Plants continuously produce tremendous quantities of organic matter that would otherwise accumulate near the soil surface if it were not decomposed by soil organisms. Soil organisms depend on the continuous supply of plant and animal residues as a source of energy and essential nutrients. This biological activity which continuously produces and destroys organic matter, recycles nutrients and produces humus is a major characteristic that distinguishes soils from geologic deposits.

Soil organic matter is a general term that includes everything organic --living biomass, partially decomposed organic residues and largely amorphous and colloidal substances no longer identifiable as tissue. The latter material is referred to as ***humus***.

Humus is formed by microbial decomposition and synthesis reactions. It includes ***humic*** and ***non-humic substances***. The non-humic substances are identifiable biomolecules such as proteins, hemicelluloses, cellulose, fats, waxes and lignins. Humic substances include ***fluvic acid***, ***humic acid*** and ***humin***. Fulvic acid has the lowest molecular weight and least resistance to decomposition by microorganisms. Humin has the highest molecular weight and greatest resistance to decomposition.

Humus is very dark in color. It has a large number of negative charges per unit mass, thus, very high CEC. The large molecules of humus bind to clay particles and greatly increase aggregate formation and stability. Physical as well as chemical soil properties of soils are greatly improved by humus.

This laboratory introduces an abbreviated method for the extraction and fractionation of humus. The alkaline extraction procedure allows separation of fulvic acid, humic acid and humin. Whereas fulvic and humic acids are extracted by base, humin is not. Fulvic acid is separated from humic acid by subsequent precipitation of humic acid with acid.

The organic soil sample is pretreated with HCl. You will qualitatively test the acid wash for the presence of Ca^{2+}, thereby demonstrating cation exchange. You will also induce flocculation and dispersion.

If cation exchange sites of humus are primarily occupied by Ca^{+2} and Mg^{+2}, humus particles remain flocculated and bind soil particles together. But if exchange sites are largely saturated with Na^{+}, the soil pH is high and humic colloids are dispersed. Under these conditions humus loses its stabilizing influence, and may be leached from the soil as individual particles or rise to the surface of the soil as water moves upward during evaporation. It is deposited there, resulting in what is called a "***black alkali***" soil.

III Procedure

Extraction of Humus

1. Weigh 1 g of an organic soil and transfer to a funnel lined with filter paper. Set a test tube under the funnel.

2. Slowly add 3 mL of HCl to the organic soil. Pour leachate through the soil.

3. Wash the soil with three 5 mL increments of distilled H_2O.

4. Collect about 1 cm of leachate in the first test tube, then move the funnel to a second test tube. Save leachate in the first tube for *Test for Exchanged* Ca^{2+} (below).

5. Once the distilled H_2O has drained from the soil, add 5 mL of 1:5 NH_4OH.

6. When the leachate begins to show a dark brown color, move the funnel to a third test tube and collect the leachate. Discard leachate in the second tube.

7. Wash soil with distilled H_2O until there are 5 cm of dark brown leachate in the third test tube.

8. Answer question # 1 below.

Flocculation

9. Pour 1/2 of the leachate from the third test tube into another tube.

10. Dilute both samples of leachate with water to a coffee brown color.

11. Add a spatula tip full of $Ca(OH)_2$ powder to one tube, shake well, and let stand.

12. Put a short piece of litmus paper in the other tube and add dilute HCl drop by drop until the leachate is acidic (litmus turns red).

13. Let stand and observe the results. Use this tube in the next section.

14. Answer questions # 2.

Dispersion

15. Add dilute NaOH dropwise to the acidified leachate from section *Flocculation of Humus* (above) until it is alkaline.

16. Shake and let stand.

17. Answer question # 3.

Test for Exchanged Ca^{+2}

18. Add 1:2 NH_4OH slowly, with constant shaking to the first test tube containing the HCl leachate until the leachate is alkaline. A light brown precipitate of iron and aluminum hydroxides will develop. Avoid an excess of NH_4OH.

19. Filter the contents of the test tube into a clear test tube.

20. Add 5 drops of ammonium oxalate to the filtrate. Watch for a white precipitate to occur.

21. Answer question # 4.

IV Questions

1. What was the predominant cation on the soil CEC after step 3 of the extraction procedure? After step 7?

   What caused the dark brown color in the leachate in step 6?

2. Did flocculation of the humus occur? If so, in which tube or tubes?

   Describe the difference in appearance of Ca-saturated and H-saturated humus.

3. Describe the appearance of humus after the NaOH was added.

4. What was the white precipitate that formed?