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 ON  
 EXPERIMENTAL STUDIES FOR THE  
 DETECTION OF PROTEIN IN TRACE AMOUNTS

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Research during this reporting period has been on the extraction and preparation of organic materials from soil and the interaction of these materials with the thiocarbocyanine dye. The approach has been to: (a) determine the tendency of inorganic polyanions which form or are stable in aqueous systems to change the dye spectrum, (b) observe the reaction of the dye with soluble and colloidal inorganic polyanions extractable from soil and (c) develop a sequence of preparative steps necessary to separate compounds of classes a and b from the polymeric organic materials extractable from soils while maintaining an optimum extraction of the organic material.

Procedures being studied for the extraction of soil organic material and the rationale for their use were reported in the previous quarterly report. The analysis of carbon values was not complete, but is now available and the results are shown in Table 1. Briefly the extraction procedure consisted of suspending the soil in the pretreatment solution for 1 hour with shaking. The soil was separated by centrifugation, washed five times with water and shaken in 0.5 N NaOH for one hour. The soil residue was separated from the extract by centrifugation. The supernate was exhaustively dialyzed against distilled water and the precipitate (extracted soil) was dried and analyzed for carbon. Several interesting points are apparent. The pyrophosphate pretreatment removed no carbon. This is an ideal result for a pretreatment, since it results in no loss of organic material. Because of this favorable result, the experiment was repeated and the previous result was confirmed. The organic carbon removed by the lactate pretreatment plus the subsequent sodium hydroxide extraction was roughly the same as that removed by the sodium hydroxide extraction of the pyrophosphate pretreated soil, but there was twice as much non-dialyzable carbon removed by the lactate system. The EDTA extraction system (pretreatment + NaOH extraction) extracted as much nondialyzable carbon as the pyrophosphate system, but only a little more than half as much total carbon from the soil. The organic carbon removed by the various pretreatments was not subjected to dialysis so it is not apparent how much was nondialyzable. However, by definition the carbon removed by 0.1 M HCl must be fulvic acid since it is soluble in acid. All pretreatments were carried out at acid pH and therefore anything removed by these solutions would also be considered fulvic acid.

The results show that with the exception of pretreatment with water, each of the pretreatment schemes was effective in increasing the amount of nondialyzable organic material extracted by 0.5N NaOH. The results with pyrophosphate demonstrate that a pretreatment can be used which will not remove organic material from the soil, but will aid in increasing the amount of material subsequently extracted with alkali. It was anticipated that this would be the type of result obtained with the HCl pretreatment, on the basis of the results obtained by Choudhri and Stevenson (Proc. Soil Science Soc. of Amer., 21, 508-513 (1957) ). Their results for four clay type soils indicated HCl pretreatment greatly increased (3 fold in one case) the humic acid solubilized, but did not greatly increase the total carbon extracted. The comparison of our results with theirs points up the problems encountered in attempting to devise a universal soil extraction procedure.

The main point to be made as a result of the foregoing work is the appropriateness of a pretreatment prior to alkali extraction. This work is now being extended to include similar studies with desert type soils. It was felt that clay containing soils offer the greatest difficulties in the extraction of organic materials and also for interference with the dye reaction. It was also felt that work with the clay soils would serve as a reference for later work with desert soils.

Adopting the premise that a pretreatment will be included in any soil extraction scheme adopted greatly simplifies the problem of exclusion and removal of inorganic materials that will interfere with the dye reaction. Previous studies of the dye reaction with various inorganic materials were presented in the last quarterly report. These studies were concerned with the removal of these materials by ultrafiltration and dialysis. These studies were repeated with the inclusion of an acid extraction and water wash prior to 0.5N NaOH extraction. The advantage gained by the acid and water wash is the removal of such polyanions as metaphosphate, isopoly and heteropoly acids. The materials studied were chosen because of their interference with the dye reaction in any of several ways, formation of polymeric species in aqueous solution, suspension as colloids, difficulty in dialysis, greater solubility

in alkali and lower solubility at neutral pH. Test materials which could be eliminated from concern because of their solubility at a concentration of 2 gm per 10 ml of 0.1N HCl were potassium pyrosulfite, potassium pyrosulfate sodium arsenate, sodium pyrophosphate, sodium metaphosphate, sodium ortho phosphate, phosphotungstic acid and sodium hyposulfite. In many cases it was necessary to add concentrated acid to overcome the buffering of the test materials and obtain a pH of at least 1.0 during the acid pretreatment. Of the materials studied only alum ( $AlK(SO_4)_2$ ) was not soluble in acid, but was dissolved in the subsequent water wash. Materials not soluble in the acid extraction or subsequent water wash were subjected to alkaline extraction. The alkaline extract was divided and one aliquot was dialyzed against water and the other against 0.1N alkali and then distilled water. Extracts from potassium persulfate, sodium arsenite, ferric oxide, poly sulfide, aluminum hydroxide and plumbous hydroxide gave no dye reaction after either water or alkali and water dialysis. Extracts from kaolin, bentonite, uralite, sodium silicate ( $Na_2O:SiO_2$ , 1:2.86), potassium silicate ( $K_2O:SiO_2$ , 1:2.10) and sand caused a change in dye spectrum after exhaustive water dialysis, but not after alkali dialysis followed by water dialysis. Phlogopite was the only aluminosilicate of the four tested (kaolin, bentonite and uralite) which still gave a positive test after the complete treatment. Because of the ubiquity of aluminosilicates in soils, it was felt that these experiments should be repeated several times to establish the reproducibility of the results. This has been done and the results were found to vary with time of dialysis. The soluble silicates could be removed with water dialysis if the dialysis period was sufficiently long. In the case of some of the aluminosilicates acid extraction prior to the alkaline extraction appears to be required to eliminate them by dialysis.

The inorganic materials chosen for this study constitute a broad spectrum of inorganic chemical types which could offer difficulties to the dye detection system. However, this present work has shown that these materials can be removed by a process of extraction which would be required in any event to free soil organic material from inorganic constituents. Because of the ubiquity of the aluminosilicates in surface materials, it is felt further studies should be carried out concerning their removal from these soil extracts.

In the case of the phlogopite preparation it is necessary to determine why it is not as easily eliminated as are the other aluminosilicates studied. The positive dye reaction obtained with the alkaline dialyzed phlogopite can not be a result of reaction with extracted organic carbon. Organic carbon analysis on all four of the aluminosilicates did not indicate a trace of carbon in these minerals.

It must be noted that the organic material extracted from the standard clay soil gives the same dye spectrum after alkaline dialysis as with the water dialysis alone. This is in contrast to the results with the aluminosilicate extracts.

#### PLANS FOR NEXT PERIOD:

Large scale extractions of the clay soil (used as a standard) and desert soils will be carried out with the proposed extraction procedure (i.e., acid pre-extraction, water wash and alkali extraction). The dialyzed extraction product will be subjected to infrared analysis to determine the relative concentration of functional groups present in these materials. The elimination of silicates from these products will be established. Selective removal of the carboxyl groups will be carried out. Reaction of the dye with the decarboxylated product and with the untreated material will be compared. To establish the nature of the dye reaction. Partial oxidation and reduction of the soil extract material will be attempted to determine its effects on the dye reaction.

Studies will be carried out in which the aluminosilicates, kaolin, bentonite, uralite and phlogopite will be subjected to the extraction procedure. The alkaline extract will be dialyzed for various periods against alkali to determine the time required to remove the aluminosilicates. Smaller pore membrane filters will be employed with the phlogopite extract to determine if this will improve its subsequent dialysis rate.

TABLE 1

## EXTRACTION OF ORGANIC CARBON FROM A CLAY SOIL

<u>Pre-treating Agent</u>	<u>% Carbon removed by pre-treatment (+ washes)</u>	<u>% Carbon removed by 0.5N NaOH (+ washes)</u>	<u>% Non-dialyzable carbon removed by NaOH</u>
None	---	18	2.96
H <sub>2</sub> O	None	34	1.43
.1M HCl	21	15	3.15
.25 M lactic acid (pH 4.3)	15	40	10.4
.1M EDTA* (pH 4.5)	9	20	4.90
.1M Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> (pH 4.5)	None	54	4.80

Soil employed in these studies was a local agricultural clay containing soil. The organic carbon content of this soil is 0.85 percent.

\* Ethylenediaminetetraacetic acid