

NOTE

HEAVY METAL AND ORGANIC
CARBON FRACTIONATION IN
SODIUM PYROPHOSPHATE
EXTRACTS OF TIDAL MARSH SOILS

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ABSTRACT

Dissolved organic carbon (DOC) and associated heavy metals in sodium pyrophosphate extracts of metal-enriched soil were fractionated using dialysis and results were compared with fractionation of dissolved organic carbon with acidification method used to separate humic acid (HA) and fulvic acid (FA). Four soil samples selected from two different sites and at two different depths of Baltimore Harbor (Baltimore, MD, U.S.A.) and Smithsonian (MD, U.S.A.) tidal marshes were extracted with 0.1 M sodium pyrophosphate. Three different methods (i.e. dialysis, acidification and inorganic salt addition) were used to fractionate the HA and FA fractions. With the exception of one soil sample, the percentage of DOC in all soils was fairly close (from 4.5% to 43% and from 11% to 40% of total DOC in acidified and dialyzed method, respectively).

The percentage of Zn and Ni remained inside the dialysis membrane were 16%-70% and 55%-84%, respectively, whereas those of Fe, Pb, Cu, and Cd were 10%-36%, 7%-28%, 5%-25%, and 14%-44%, respectively. Thus, most of Fe, Pb, Cu, and Cd were

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associated with the FA, whereas Zn and Ni were mostly associated with HA or any organic substance which had not passed through the dialysis membrane (m.w. cutoff 3500). Addition of ammonium sulfate and cesium nitrate produced no precipitation.

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جداسازی فلزات سنگین و کربن آلی خاکهای باتلاقی در عصاره پیروفسفات سدیم

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چکیده

کربن آلی محلول و فلزات سنگین پیوسته بدان در خاک غنی از فلزات در عصاره پیروفسفات سدیم به روش دیالیز جدا سازی شدند و نتایج حاصله با نتایج حاصل از جدا سازی کربن آلی محلول به روش اسیدی کردن بمنظور جدا ساختن اسید هومیک و اسید فولویک مقایسه گردید. عصاره چهار نمونه خاک باتلاقی از دو عمق متفاوت و دو محل مختلف در ساحل بالتیمور (ایالت مریلند، آمریکا) و اسمیتسونین (ایالت مریلند، آمریکا) در محلول ۰/۱ مولار پیروفسفات سدیم تهیه گردید. سه روش مختلف (از قبیل دیالیز، اسیدی کردن و افزایش نمکهای معدنی) بمنظور جدا سازی اسید فولویک و اسید هومیک مورد استفاده قرار گرفت. با استثنای یک نمونه از خاکها، مقدار کربن آلی محلول در همه خاکها نسبتاً نزدیک بهم بود (۴۳-۴/۵% و ۴۰-۱۱% مجموع کربن آلی محلول بترتیب در روش اسیدی کردن و دیالیز). مقدار روی و نیکل بجا مانده درون غشای دیالیزی به ترتیب ۷۰-۱۶% و ۸۴-۵۵% بود در حالی که مقادیر آهن، سرب، مس و کادمیم بترتیب عبارت از ۳۶-۱۱%، ۲۸-۷%، ۲۵-۵% و ۴۴-۱۴% بودند. بنا بر این بیشتر مقادیر آهن، سرب، مس و کادمیم پیوسته به اسید فولویک، در حالی که روی و نیکل بیشتر پیوسته به اسید هومیک یا هر ماده آلی بودند که از غشای دیالیز (m.w. cutoff 3500) عبور نکرده بودند. با افزایش سولفات آمونیم و نیترات سزیم هیچگونه رسوبی حاصل نشد.

INTRODUCTION

Water quality in the Chesapeake Bay (eastern coast of the U.S.) and its tributaries has been declining steadily during the mid and late 20th century. Most of these problems appear to be related to increased nutrients such as P and N along with increased amounts of toxic materials (3).

Heavy metals have been identified as toxic pollutants at low concentrations. The behavior of heavy metals is usually seen in context with precipitation and organometallic complexation reactions in the environment.

The complex formation of metallic cations with HA and FA fractions of soil organic matter is of particular importance since they play an important role in plant nutrition and metal mobility in soil (1). Hahne and Kroontje (1) have pointed out that in areas where organic matter accumulation takes place, organo-metal-ion complexes are formed which may exist in soluble or colloidal form and, therefore, differ in degree of mobility.

In order to separate different fractions of organic material and to study the associated heavy metals, it is first necessary to separate the organic substances from soil samples. The major difficulty in all investigations relevant to composition of soil humus is that it can neither be separated from unhumidified matter nor from the mineral constituents of the soil. The methods used, separate out only a part of the humus, and some may alter the original constituents of the humus into forms that disperse easily (4). Since the humus is bound to the clay through polyvalent cations or through specific adsorption, treatment with a complexing ligand such as pyrophosphate will be an effective dispersing agent for soil organic matter and metallic complexes (4, 6).

The proportion of the humic matter that is precipitated by the acid depends on the type of acid used and its strength (4). The acidification may be a suitable method for fractionating humic substances, but with regard to studying organo-metal complex, there might be some weakness such as extraction of other portions of metals in soil. The fractionation could be easily accomplished by adding a salt instead of acid, particularly if the alkali is first removed with a suitable H⁺saturated exchange resin (4).

The dispersed humic and fulvic particles having a wide range of molecular weights are analytically difficult to be separated into fractions (4). It is possible to separate some low-molecular weight HA and FA during the separation process. Although dialysis is a widely-used technique for inorganic salt removal from solutions, a low-molecular-weight components of FA might be lost through the dialysis tubing (2). This loss may be serious because of the chemical heterogeneity of FA (2).

Elemental chemical and infrared spectral analyses demonstrate that the eluted fulvic components are different from the bulk of FA that did not move through the dialysis tubing. In particular, the infrared spectra indicate that the low-molecular weight fulvic components contain significantly more COOH groups than the FA retained inside the tubing. These components are just the ones that are more reactive with trace metals, since COOH groups have been suggested to be important in metal complexation (2). We hypothesized that the procedure could be used successfully to separate the FA and HA along with associated heavy metals. Based on this hypothesis, the objectives of the study were (i) to fractionate dissolved organic carbon (DOC) and associated heavy metals in sodium pyrophosphate (Na₄P₂O₇) extracts using dialysis and (ii) to compare the results with fractionation of DOC using acidification method for separation of HA and FA.

MATERIALS AND METHODS

Four soil samples were selected from two different sites and at two different depths of Baltimore Harbor (Maryland, U.S.A.) and Smithsonian tidal marsh (Maryland, U.S.A.) (Table 1). All samples were freeze-dried for 48 hr and then crushed. Five grams of each sample were placed into a 100 ml propylene centrifuge tube and 50 ml of 0.1 M sodium pyrophosphate adjusted to pH 7.0 was added. The samples were shaken at room temperature for 24 hr without displacing the air in tubes with N₂ and then centrifuged at 6000 rpm (RCF=4000) for 10 min according to Schnitzer (6). Some modifications were used i.e., freeze drying instead of

Table 1. Soil identification (Griffin 1987, unpublished data).

Soil sample	Site	Depth (cm)	Place	Organic Loss on	
				C (%)	ignition (%)
1	5	20-30	Baltimore Harbor tidal marsh	23.81	37.5
2	5	40-50	Baltimore Harbor tidal marsh	1.92	8.0
3	8	15-20	Smithsonian tidal marsh	11.54	27.8
4	8	60-70	Smithsonian tidal marsh	47.44	98.8

air drying; shaking under air rather than N₂; and centrifuging at 6000 rpm instead of 10,000 rpm. The centrifugates were then passed through the ashless filter paper (Whatman No. 40). By adding 5 ml of distilled water to each tube, centrifugation and filtration were repeated. Because of some turbidity all samples were ultracentrifuged at 10,000 rpm (RCF=11,100) at 20°C for 30 min. The clear supernatant liquids were analyzed for Fe, Zn, Cu, Pb, Ni, and Cd by atomic absorption spectrophotometry. Five ml of each extract were also acidified with 3.8 ml of 0.1 M sulfuric acid to bring the pH to about 2.0, then kept at room temperature for 24 hr. After that, all acidified samples were centrifuged at 6000 rpm (RCF=4000) for 30 min. The supernatants were collected, and the amount of the DOC was measured following UV light-enhanced persulfate oxidation and IR-detection of CO₂ using a Xertex-Dohrmann DC-80 carbon analyzer. Dissolved organic carbon was also measured on original extract of the soils to estimate the sum of HA and FA in the extracts. To operationally define HA and FA in the extracts, 10 ml of each sample were put into a 5-cm dialysis tubing (m.w. cutoff 3500), placed in a plastic cup containing distilled deionized water, and put on a rotary shaker (200 rpm) for a week at 25 ± 2°C. The distilled water was changed every day. After one week, the concentration of DOC and Fe, Zn, Cu, Pb, Ni and Cd remaining in the dialysis tubes were measured.

In order to fractionate the humic substances (HA and FA) without any alteration phenomenon (i.e. acidification), and also to compare with the dialysis method, 1 ml of one of the four extracts (site 5/20-30) was taken, and 40 drops of 4.1 M ammonium sulfate were added, and then passed through a PC membrane (diameter = 25 mm and pore size = 0.4 μm). The same procedure was followed using cesium nitrate.

RESULTS AND DISCUSSION

Comparing the amount of DOC of the acidified and dialyzed HA, the results indicate that, except for soil No. 2 in which the difference between the acidified and dialyzed HA is relatively high (16% vs 40% of the total organic carbon), in other soils the amounts of DOC are fairly close (Fig. 1). The amounts of acidified and dialyzed HA, in terms of percentage of the total organic carbon, ranged from 4.5% to 43% and from 11% to 40%, respectively (the numerical results of this comparison are not shown in this paper). Table 2 shows the comparison of DOC in four soil samples before and after dialysis and the ratio of these amounts. Table 3 reveals the above results along with the amounts of associated heavy metals. As it is shown in this table, the total amount of all metals in the upper horizons of soils No. 1 and 3 are higher than that of the lower horizons. The results also show that most of the Fe had passed through the dialysis membrane. The concentration of Fe remained inside the membrane ranged from 10% to 36%, while the percentage of DOC remained inside the membrane varied from

Table 2. Dissolved organic carbon in four different soil samples before and after dialysis.

Soil sample	DOC (mg kg^{-1})		A/B(%)
	B	A	
1	13800	4900	35
2	2500	1000	40
3	8800	700	11
4	2200	700	32

B = Before dialysis.

A = After dialysis.

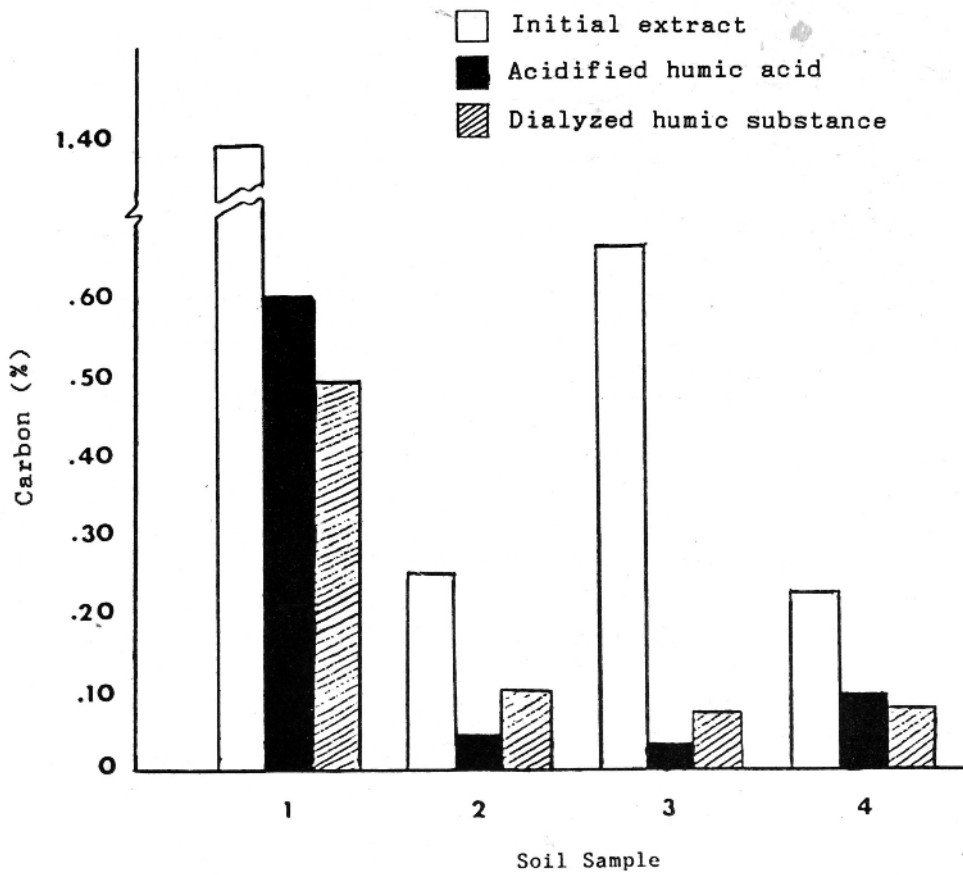


Fig. 1 Comparison of percentage of carbon in the initial extract (total carbon), acidified humic acid and dialyzed humic substance in the four soils.

Table 3. Amount of heavy metals and dissolved organic carbon (DOC) in four soil extracts before and after dialysis, and the ratio of these amounts in term of percentage.

Soil sample	Fe	Zn	Cu	Pb	Ni	Cd	DOC
1:							
B† (mg/kg)	3448.5	132.00	50.05	88.55	8.18	0.83	13800
A‡ (mg/kg)	560.1	22.00	12.10	5.28	5.17	0.37	4900
A/B (%)	18	18	24	8	84	44	35
2:							
B (mg/kg)	888.8	11.55	4.35	7.48	3.38	0.43	2500
A (mg/kg)	323.4	8.18	0.22	0.68	1.87	0.08	1000
A/B (%)	36	53	5	9	55	14	40
3:							
B (mg/kg)	818.4	41.25	3.03	11.17	3.08	0.22	8600
A (mg/kg)	80.0	11.88	0.77	0.77	2.20	§	700
A/B (%)	10	29	25	7	71	§	11
4:							
B (mg/kg)	107.8	9.80	0.50	0.39	§	§	2200
A (mg/kg)	12.3	8.84	0.11	0.11	1.78	§	700
A/B (%)	1	70	22	28	§	§	32

†B = Before dialysis.

‡A = After dialysis.

§No detectable amount.

11% to 40%. It seems that most of the Fe in the soils is associated with FA. The Pb and Cd (especially in the lower horizon of Smithsonian site which was not detectable) are mostly associated with FA or any organic substance which had passed through the dialysis tubing. The percentage of Pb

remained inside the membrane is from 7% to 28%, whereas that of Cu varies from 5% to 25%. The relatively high percentage of Cu removed by FA may have been related to forming relatively stable water-soluble FA-Cu complexes. Furthermore, it could be attributed to high complexing power of FA for Cu⁺⁺, especially at pH above 3.5 (5). The results also indicate that Zn and Ni are mostly associated with the HA and/or their structural complexing arrangement and thus they did not pass through dialysis tubing used in the present study. As Table 3 shows in soil No. 1, 35% of total DOC and 84% of Ni, and in soil No. 3, 11% of total DOC and 71% of Ni remained inside the membrane. In general, the amount of Ni in the Baltimore site is higher than the Smithsonian site. This may be due to the fact that Baltimore is an industrial area disposing this metal into the environment, whereas Smithsonian is located in the southern part of Annapolis which is not as industrialized as Baltimore.

Addition of ammonium sulfate and cesium nitrate produced no precipitation. In order to confirm this failure, these humic substances were acidified and the flocculation phenomenon was observed. This might be due to the fact that because of relatively high amounts of Na very strong dispersing agent present in the solution, the salts added to the humic substance were not able to flocculate the dispersed solution.

CONCLUSION

Comparison of the amounts of DOC of the acidified and dialyzed HA, indicates that the dialysis method could be an appropriate approach in order to separate HA and FA without acidifying the organic substance and consequently altering the organo-metal complex structure.

With the exception of Ni and Zn, especially in the lower horizons, most of the metals were

associated with FA. The fact that FA is a highly stable water-soluble organic substance demonstrated this characteristic might be beneficial from the standpoint of agriculture and plant nutrition. However, the problem of water quality and environmental safety should also be considered because it seems that the stability of the organo-metal complexes cannot prevent the harmful effects of the associated metals.

Finally, it is suggested that a similar study may be initiated by using Ca^{++} or Ba^{++} salts. Based on the lyotropic series, the Ca and Ba salts are stronger in flocculating dispersed solutions than the Cs and NH_4^- salts.

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