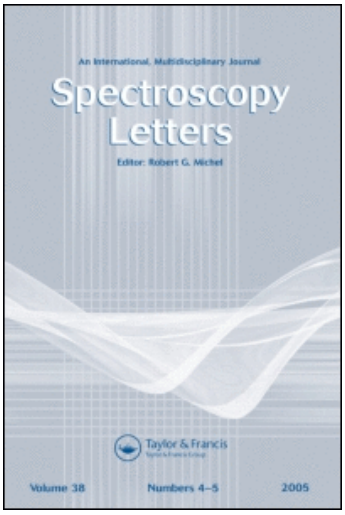

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Spectroscopic Analysis of Sequentially Extracted Humic Acid from Compost

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Abstract: Structural characterization of organic matter (OM) is crucial in assessing its role in soils and sediments. In this study, humic acids (HA) were sequentially extracted from a compost and characterized in terms of their molecular, chemical, and structural heterogeneity. HA was extracted in sequence a total of 28 times, using 0.1 mol L^{-1} $Na_4P_2O_7$, and sequentially grouped into seven fractions. These fractions and the original compost were analyzed using UV–visible spectroscopy, ^{13}C nuclear magnetic resonance spectroscopy (NMR), diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), and an elemental analyzer. ^{13}C-NMR and DRIFTS data indicated significant structural variations among the extracted HA fractions and the original compost. These data corresponded to a decrease in the atomic C/H ratio and to an increase in the atomic C/O ratio among these sequentially extracted HA fractions, which together represented an increase in aliphaticity in conjunction with a decrease in polarity and aromaticity. E_4/E_6 ratios indicated an increase in molecular size with increasing number of extractions. The structural variability of HA may significantly affect the sorption of organic pollutants and the reduction and complexation of metals by organic matter.

Keywords: ^{13}C-NMR, DRIFT, E4/E6 ratio, humic acid, sequential extraction

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INTRODUCTION

Compost is an end product of degraded organic matter (OM) and consists of transformed, slowly degradable compounds, intermediate breakdown products, and dead microorganism cell walls that are classified as humic substances (HS).[1] Increasing the organic matter content of soils improves aeration, moisture retention, soil structure, and has been found to reduce plant disease and control weeds more efficiently than commercial fertilizers and herbicides.[2] Organic matter must be characterized in order to predict and understand its behavior and chemistry. Numerous studies have attempted to decipher its chemical composition, structure, and conformation; however, due to the characteristic heterogeneity of samples, there is no definitive structure for organic matter, although many structural models have been developed.

HS are composed of three major fractions: base soluble humic acid (HA), fulvic acid (FA), soluble at any pH, and humin, insoluble at all pH levels. Past studies have attempted to elucidate the structure of HA based on a single extraction process; however, this method provides only a broad overview of the general structure of HA and may omit significant details due to the aggregate nature of samples. The objective of this study was to observe any structural changes occurring among the HA fractions extracted in sequence from compost. Individual fractions were examined with a variety of spectroscopic techniques in order to assess the qualitative changes in the various fractions.

MATERIALS AND METHODS

In this experiment, compost was made from food waste at the University of Massachusetts, Amherst. The compost was rinsed free of debris with water, air-dried for 7 days, and passed through a 2-mm sieve. Then, 100 g of compost was placed into each of two 1-L polyethylene bottles, and 1000 mL of $0.1\,\text{mol}\,\text{L}^{-1}$ $Na_4P_2O_7$ solution was added to each bottle to attain a soil: solution volumetric ratio of 1 : 10, which was maintained throughout the experiment. The air in the bottles was replaced with N_2 gas, and the mixtures were shaken for 24 hr at room temperature. After mixing, the suspension was centrifuged at $3000 \times g$ for 15 min. The supernatant was collected as the first extraction, and the residue was rinsed back into the two bottles with $Na_4P_2O_7$ solution to reestablish a 1 : 10 ratio. The residue was extracted a total of 28 times with the $0.1\,\text{mol}\,\text{L}^{-1}$ $Na_4P_2O_7$ solution. HA fractions were made by combining the extractions and acidifying the suspensions to pH 1.5 each time a new extraction was added, until the amount of precipitate reached an amount adequate to produce an HA sample for analysis. The corresponding extraction numbers for each HA fraction are presented in Table 1. The acidified HA precipitate was collected by centrifugation at $5000 \times g$

Table 1. Extraction sequence and corresponding group fraction assignment for humic acids

HA fractions	Number of extractions
F-1	1, 2
F-2	3, 4
F-3	5, 6, 7
F-4	8, 9, 10, 11
F-5	12, 13, 14, 15, 16
F-6	17, 18, 19, 20, 21, 22, 23
F-7	24, 25, 26, 27, 28

for 30 min and labeled as a fraction (e.g., F-1, F-2). Each fraction was de-ashed 3 times with 0.1 mol L^{-1} HCl/0.3 mol L^{-1} HF solution, rinsed 3 times with deionized water, freeze-dried, gently ground, and set aside for analysis.

An Agilent 8453 UV-visible spectrophotometer (Palo Alto, CA, USA) was used to obtain spectra for all HA fractions, which were dissolved separately in 0.1 mol L^{-1} NaOH and then diluted with 0.05 mol L^{-1} $NaHCO_3$. The E_4/E_6 ratio reflects the ratio of the absorbance at 465 nm to that at 665 nm.[3,4]

Elemental analysis was performed by a microanalytical laboratory at the University of Massachusetts, Amherst. C, N, O, and H percentages were obtained for all seven HA fractions. The instrument used was an Exeter Analytical 240XA Elemental Analyzer (North Chelmsford, MA, USA). Ash content was measured by heating the samples at 800°C in a muffle furnace for 4 hr.

Fourier transform infrared spectroscopy (FTIR) was performed using a Midac series M 2010 infrared spectrophotometer (Irvine, CA, USA) with a DRIFTS accessory (Spectros Instruments, Whitinsville, MA, USA). KBr was mixed with each sample; each sample was then ground with an agate mortar and pestle, transferred to a sample holder, and smoothed with a glass microscope slide. N_2 gas was used to flush the diffuse-reflectance cell holding the samples for 30 s prior to each reading to eliminate interference from CO_2 and ambient moisture. One hundred scans were collected at a resolution of 16 cm^{-1}, and the spectra with numerical value for major peak wave-numbers and intensities were recorded.[5,6] We examined the spectrum between 400 and 4000 cm^{-1}.

Solid-state cross-polarization magic-angle-spinning and total-sideband–suppression (CPMAS-TOSS) ^{13}C-NMR spectra were obtained using a Bruker DSX-300 spectrometer (Karlsruhe, Germany) operated at the ^{13}C frequency of 75 MHz. The instrument was run under the following condition: contact time, 2 ms; spinning speed, 5 kHz; 90° ^{1}H pulse, 5 μs; acquisition delay, 4 s; and number of scans, from 5000 to 10,000.[7]

All ^{13}C-NMR spectra were divided into five regions,[3] and integrated as follows: aliphatic and paraffinic carbon (0–50 ppm); methoxy groups, oxygenated aliphatic carbon, and anomeric carbon (50–110 ppm); aromatic and phenolic C (110–160 ppm), carboxylic groups (160–190 ppm); and carbonyl and ketonic groups (190–220 ppm). Percentage ratios were calculated by integrating the area under each spectral curve for the five separate regions. Total aromaticity was expressed as a ratio of aromatic C (110–160 ppm) divided by the sum of aliphatic C (0–110 ppm) and aromatic C.[1]

RESULTS AND DISCUSSION

No previous study, to our knowledge, has used such an extensive extraction process (i.e., 28 times) for HAs as our own, the significance being that HS may have highly aggregated and complex structures, the bonds of which may require significant disturbance to break,[3] thus, a large number of extractions. Aliphatic material, for example, may appear more significantly in later extractions of HA than aromatic material. This is shown by the results of this research, as well as three recent studies.[7–9] Gunasekara and Xing[10] demonstrated the contribution of aliphatic moieties to the high sorption capacity and nonlinearity for organic contaminants. Using $Na_4P_2O_7$ allowed us to extract less HA at one time than traditionally used NaOH. We were therefore able to produce small, sequential fractions, which could be thoroughly examined for structural variations.

Elemental compositions for all seven HA fractions are summarized in Table 2, and indicate significant chemical heterogeneity among the extracted HAs. The contents of C increased to a slight degree overall from 46% in F-1 to 51% in F-7, and the content of H increased from 4% in F-1 to 6% in F-7. O content decreased from 43% for F-1 to 37% for F-7. The N content remained fairly constant among all HA fractions. The C/H atomic ratio

Table 2. Elemental compositions, atomic ratios, and ash contents of humic acid fractions from F-1 to F-7

Sample	C (%)	H (%)	N (%)	O (%)	Ash (%)	C/H	C/O
F-1	46	4.0	5.2	43	1.3	0.95	1.4
F-2	51	4.5	5.6	38	0.8	0.95	1.7
F-3	51	5.0	5.8	37	0.5	0.85	1.8
F-4	52	5.5	5.9	36	0.7	0.80	2.0
F-5	53	6.0	6.1	34	0.6	0.74	2.1
F-6	52	5.6	5.5	36	0.8	0.78	1.9
F-7	51	6.0	5.4	37	1.1	0.71	1.8

C/H, atomic ratio of carbon to hydrogen; C/O, atomic ratio of carbon to oxygen; F-1 to F-7 are humic acid fractions (see Table 1).

decreased from 0.9 for F-1 to 0.7 for F-7, and the C/O atomic ratio increased from 1.4 for F-1 to 1.8 for F-7. The changes in the atomic ratios from F-1 to F-7 suggest that the HAs became more aliphatic and less polar with progressive extraction. Ash content was relatively low for all HA fractions, ranging from 0.5% to 1.3%.

The ^{13}C-NMR spectra of HA fractions 1, 2, 6, and 7 are shown in Fig. 1 and the percentages of the integrated peak regions are shown in Table 3. The molecular heterogeneity among HA fractions is apparent from the ^{13}C-NMR spectra, which changed gradually over the course of extraction. Major peaks for the HA spectra are present at 30–33 ppm (methylene C), 56 ppm (methoxy C), 70 ppm (carbohydrates), 128 ppm (C-substituted aromatic C), 150 ppm (O-substituted aromatic C), and 171 ppm (carboxyl C). The HA spectra shared comparable features with those analyzed in other HA studies.[7,9]

Peak intensities in the region from 0 to 110 ppm increased and became more resolved from F-1 to F-7, reflecting an increase in total aliphatic carbons with progressive extraction. Peak percentages for the HAs in the paraffinic C region (0–50 ppm) increased from 14% in F-1 to 29% in F-7. A peak

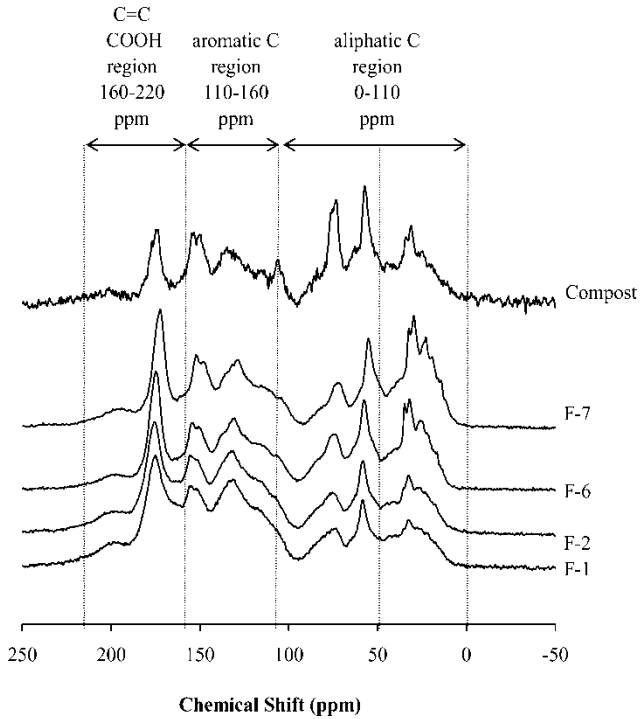

Figure 1. Solid-state ^{13}C-NMR spectra for humic acid fractions 1, 2, 6, 7, and the original compost.

Table 3. Integration of solid-state ^{13}C-NMR spectra of selected HA fractions and the original compost

	Distribution of C chemical shift (ppm), %					
Sample	0–50	50–110	110–160	160–190	190–220	Aromaticity
F1	13.6	18.6	40.3	21.4	6.59	0.56
F2	16.1	20.0	38.5	19.9	5.81	0.52
F6	22.4	25.2	34.1	16.7	1.78	0.42
F7	28.0	19.6	32.7	16.5	3.15	0.41
Compost	23.4	34.3	30.3	10.4	1.60	0.34

Aliphatic region, 0–110 ppm; aromatic region, 110–160 ppm, aromaticity = aromatic C (110–160 ppm)/[aliphatic C (0–110 ppm) + aromatic C].

representing amorphous aliphatic carbon (30 ppm) became increasingly sharp and well resolved over the course of extraction, at the expense of crystalline carbon (33 ppm). This result, obtained from a compost, is somewhat inconsistent with findings by Li et al.,[9] who used a peat soil, and with those by Kang et al.,[7] who used a forest soil, both of whom observed that the intensity of crystalline aliphatic peaks increased with progressive extraction. Hu et al.[11] suggested that crystalline aliphatic carbons may be more resistant to degradation and environmental changes than amorphous C (30 ppm), and therefore have longer residence times in soils. The evolution of a broad, unresolved peak at 30–35 ppm to a sharp, well-resolved peak, such as exhibited by the NMR spectra from F-1 to F-7, has been found to represent a transition from a random mixture of paraffinic carbons to a paraffinic mixture having finite molecular configurations.[12] Salloum et al.[13] identified amorphous and crystalline regions in the spectra of cuticular waxes. These waxes are known to derive from the plant cuticular materials such as cutin or suberin, are resistant to microbial and chemical degradation, and have limited solubility in aqueous solution,[14] which may help to explain their extended residence times in soils and sediments.[7]

Spectra in the oxygenated aliphatic carbon region from 50–110 ppm (Fig. 1) show dominant peaks at 56 ppm and 72 ppm, which can be assigned to methoxy groups (50–65 ppm) and O-alkyl groups such as carbohydrates (65–95 ppm). Peak percentages for this region reflect an increase from 19% for F-1 to 25% for F-6, and a subsequent decrease from F-6 to 20% for F-7. Methoxy groups are believed to originate from lignin.

Three peaks identified in the region from 110 to 160 ppm at 128 ppm (aromatic C), 145 ppm and 150 ppm (phenolic C) broadened and became less resolved over the course of extraction. There was an overall decrease in the corresponding peak percentages of aromatic C (110–160 ppm), from 40% for F-1 to 33% for F-7. These data correspond to an overall decrease in aromaticity over the seven HA fractions (Table 3).

The dominant peak in the region from 160 to 220 ppm developed at 170 ppm, representing carboxyl C. Peak areas in this region decreased overall from 28% for F-1 to 20% for F-7. This concurred with the overall decline in aromatic carbon (Table 3) from F-1 to F-7, and corresponds to an overall decrease in polarity over the seven HA fractions, which is in agreement with the elemental data presented in Table 2. Overall aromaticity of all HA samples decreased by 0.15 from F-1 to F-7. For all samples, the aliphatic peak (0–110 ppm) percentages increased by 15% from F-1 to F-7, and aromatic peak percentages decreased by 8% overall from F-1 to F-7.

The DRIFT analysis also permits an investigation of solid samples with a minimum of sample processing. All seven HA fractions exhibit similar IR spectroscopic features (Fig. 2), as do both humin samples. A broad band from F-1 to F-7 in the region from 2500 to 3500 cm^{-1} represents a strong H-bonded OH absorption. A peak developed at 2962 cm^{-1} between F-1 and F-7, representing CH_2 symmetric stretching.[15] This peak became more intense with progressive extraction, representing an increase in aliphatic carbon, which is consistent with our findings using ^{13}C-NMR. A broad peak with three smaller peaks in the region from 1500 cm^{-1} to 1700 cm^{-1}

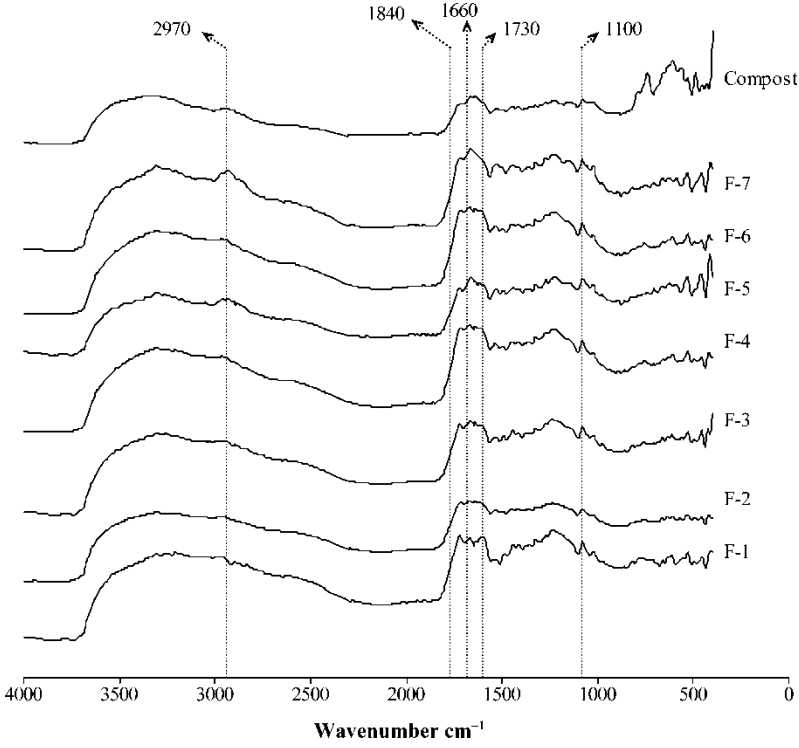

Figure 2. DRIFTS spectra of humic acid fractions and the original compost.

decreased in size over the seven HA fractions and represents a decrease in aromatic carbon groups. Carboxyl vibrations were assigned to 1710 cm^{-1}, and conjugated C═C or H-bonded C═O of carbonyl were assigned to 1630 cm^{-1}.[3,15,16] The DRIFTS results corresponded to the ^{13}C-NMR spectra, as well as to the elemental analysis, all three of which showed a decrease in aromatic C, with a corresponding increase in aliphatic C, as a function of progressive extraction.

The E_4/E_6 ratio is known to correspond to a variety of properties, notably that a decrease in the ratio reflects an increase in molecular size for HAs.[3,4] Overall, the E_4/E_6 ratio for the seven HA fractions decreased with progressive extraction (Fig. 3). We observed that F-6 and F-7, in preparation for analysis, did not dissolve as readily in solution as F-1 through F-5, suggesting the decreased solubility of the latter fractions. Khalaf et al.[17] showed that aliphatic molecules are larger in molecular size than aromatic molecules; therefore, the E_4/E_6 ratio for the HAs corresponds to the results of ^{13}C-NMR and DRIFTS spectra, which reflect an increase in aliphaticity of HAs with progressive extraction.

CONCLUSIONS

The results that we obtained in this experiment bore similarities to the results of two earlier studies involving an older forest soil and a peat soil.[7,9] An increase in aliphaticity and a decrease in polarity among progressively

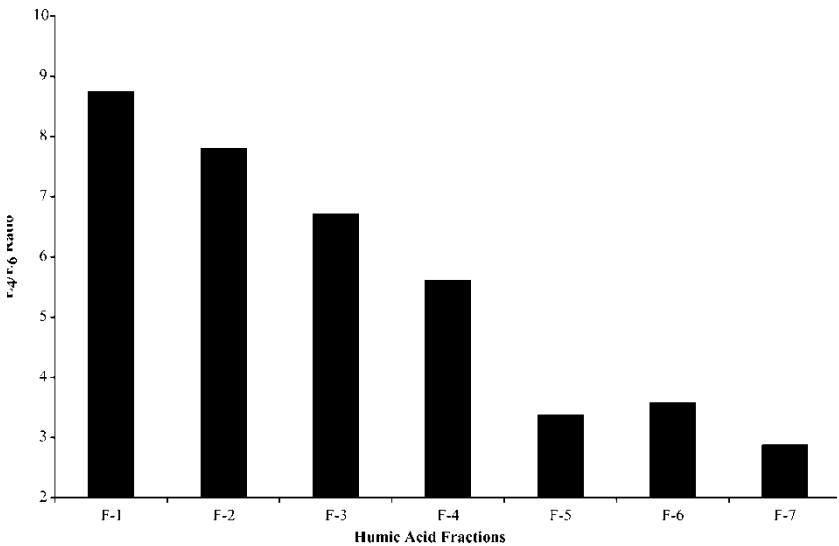

Figure 3. E_4/E_6 ratio plot for extracted humic acid fractions 1–7 from compost.

extracted HAs was common to all of the data from these studies, suggesting that the structural characteristics and variations of humic acids may be common to organic matter samples from a variety of sources. Additionally, the results of these studies agree that aliphatic structures are characteristic of HAs. As stated previously, it is known that aliphatic structures in soil organic matter contribute significantly to the increased sorption of organic pollutants. Therefore, the incorporation of compost or other organic matter into the soil may significantly affect their fate and may have significant benefits to agriculture and environmental sustainability.

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