DIVISION S-2—SOIL CHEMISTRY

Chemical and Molecular Heterogeneity of Humic Acids Repetitively Extracted from a Peat

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ABSTRACT

    Eight humic acid (HA) fractions obtained by repetitive base extraction of a peat soil were characterized in terms of chemical compositions, functionalities, and molecular sizes using an elemental analyzer, infrared spectrometry (IR), solid-state ^{13}C-nuclear magnetic resonance spectrometry (^{13}C-NMR), and high performance size-exclusion chromatography (HPSEC). Large variations in chemical, functional, and molecular properties were observed among the eight humic fractions. The elemental analysis data showed that, from the first (Fr1) to the eighth fraction (Fr8), the O/C atomic ratio decreased from 0.52 to 0.36 whereas the H/C atomic ratio increased from 1.1 to 1.5. The measured average apparent molecular size (M_w) increases from 7.7 to 22.1 kDa as a function of the extent of extraction. Carbon-13 NMR and FTIR spectra of the fractions indicated that the contents of oxygen-containing and aromatic functional groups decreased and the contents of aliphatic groups increased from Fr1 to Fr8. The results suggested that two subunits of HAs might exist: an aliphatic subunit having larger apparent M_w and an aromatic subunit having smaller apparent M_w. We proposed that each of the eight HA fractions is a mixture of these two subunits.

HUMIC ACIDS are complex organic substances formed during biogeochemical degradation of plant debris and animal residues and condensation of the degraded segments. Their amphipathic nature enables them to interact with a wide variety of inorganic and organic pollutants including heavy metals and charged organic pollutants via chemical bonding and less polar organics through nonspecific physical interactions. Because of their ubiquity in surface aquatic and groundwater systems, HAs often play important roles in environmental processes governing the fate and transport of organic and inorganic pollutants in natural systems (Weber, 1988; Bartschat et al., 1992; Stevenson, 1994). Numerous studies had shown that HAs have highly heterogeneous structures and functionalities and varied elemental compositions. While their detailed molecular structures remain unclear, HAs have long been believed to be mixtures of variously cross-linked macromolecules having heterogeneous nonrepeating structures. When dissolved in solution, these macromolecules exhibit polydispersive behavior and their dynamic molecular sizes depend largely on solution chemistry. Recent studies suggest that HAs may be aggregates of smaller heterogeneous organic molecules including sugars, organic acids, and other aliphatic and aromatic components likely having molecular weights of several hundred daltons. These small molecules may be held loosely by H bonding and hydrophobic forces instead of covalently bonded cross linkages (Piccolo et al., 2002). However, no data have shown convincingly whether HAs are cross-linked macromolecules or loosely held aggregates.

Since heterogeneity is a major factor that obscures elucidation of molecular structures of HAs, understanding chemical, structural, and molecular heterogeneity of HAs has long been a focus of soil chemistry. Previous studies employed various extraction and separation techniques to isolate HAs into fractions that are relatively homogeneous compared with bulk samples. In this study, we employed the conventional base extraction procedure to repeatedly extract HA from a given amount of soil sample until exhaustion. Instead of combining the HA extracts of different cycles as common practice, we recovered the HA of each cycle as a separate sample. The objectives of this study were to examine the differences in chemical and molecular properties among HA fractions obtained in different cycles and to investigate possible factors causing such differences. It is intuitive that the HAs could be fractionated by repetitive extraction because of the differences of solubility in alkaline solutions and affinities for solid surfaces. Humic acids having higher solubilities and lower affinities for solids should be extracted earlier. It is therefore expected that the HAs extracted in later cycles are less soluble and relatively more homogeneous, and may have larger sizes and lower polarity.

To test the above hypotheses, we systematically characterized the HA fractions extracted from a peat soil in terms of elemental composition, functionality, and apparent molecular sizes using various techniques. Our study showed that the large variations in chemical and molecular properties among the different fractions are attributable to very different extractabilities of two chemically and structurally different subunits of HAs. One subunit is aromatic and polar in nature and is highly soluble in alkaline solution whereas the other is aliphatic, less polar, and less soluble in alkaline solution. Each fraction appears to be a mixture of the two subunits in a certain ratio.

MATERIALS AND METHODS

Peat Sample

    Pahokee peat was obtained from the International Humic Substances Society (IHSS). This sample is highly humified

Abbreviations: ^{13}C-NMR, solid-state ^{13}C-nuclear magnetic resonance spectrometry; HA, humic acids; HPSEC, high performance size exclusion chromatography; IHSS, International Humic Substances Society; IR, infrared spectrometry; M_w, apparent molecular size.

and has total organic C (TOC) and ash contents of 45.7 and 15 wt%, respectively. It was selected for this study because it requires multiple cycles of base extraction for removal of the majority of the associated HAs. The sample has been widely used to study the sorption of organic and inorganic pollutants, so further elucidation of the chemical and molecular heterogeneity of its HA may provide information on the mechanisms of pollutant sorption (Lyon, 1995; Xing and Pignatello, 1997; Chiou et al., 2000; Mao et al., 2000; Xia and Pignatello, 2001).

Extraction of Humic Acids

Eight HA fractions were obtained by repeatedly extracting the soil eight times following a standard procedure recommended by IHSS (Swift, 1996). In brief, a predetermined amount of the soil was treated in centrifuge bottles with 0.1 M HCl (pH = 1–2) (1:10 w/w) to remove carbonates, Fe and Mn oxides and hydroxides. After centrifugation (2400 $\times$ g) and decanting the supernatant, base solution (1 M NaOH) was added to the soil slurry under N_2 until the solution phase became neutral (pH approximately 7), and an appropriate volume of 0.1 M NaOH was added to the bottles to achieve a soil/solution volumetric ratio of 1:10. After 24 h of soil-water contact, the soil was separated from the solution phase by centrifugation (9400 $\times$ g). The supernatant was collected, acidified with 6 M HCl to pH 1 to 2 over 20 h to allow the extracted HAs to precipitate. Again after centrifugation and decanting the supernatant, the HA precipitate was redissolved by adding a minimum volume of 0.1 M KOH under N_2, and an appropriate amount of solid KCl was added to attain an aqueous K^+ concentration of 0.3 M. The solution was centrifuged to remove fine mineral particles. The aqueous HA solution was again acidified and precipitated. After centrifugation, the precipitate was treated with 0.1 M HCl + 0.3 M HF solution for 24 h, dialyzed against distilled water, freeze-dried, gently ground, and stored until use for characterization.

The above extraction procedure was repeated eight times for the same batch of the peat until the supernatant turned slightly yellowish, suggesting HA had been exhaustedly extracted. During extraction, the volume of the base solution used and other extraction conditions were kept the same in each cycle. Nine fractions of HAs were obtained. The first eight fractions were used in this study, but the ninth fraction was not included because of its insufficient mass. The total mass of the extracted freeze-dried HAs was 49% of the mass of the original peat sample.

Characterization of Humic Acid Fractions

The C, H, N, and O contents of each HA fraction were determined with a CHN-O-RAPID Elemental Analyzer (Heraeus, Germany), following a standard high-temperature combustion procedure.

The contents of phenolic and carboxylic groups were determined for each HA fraction following a potentiometric acid/

base titration procedure (Barak and Chen, 1992). The aqueous HA solutions used for titration were at a concentration of 10 mg L^{-1} and pH of 2.8. It was free of CO_2 and had 0.1 M of NaCl as the background electrolyte. Titration was performed under an N_2 atmosphere on an automatic titrator using a standard 0.05 M NaOH solution with an increment of 0.05 mL. An aqueous solution containing 0.1 M NaCl but no HA was used as a background solution.

The IR spectra were collected at a resolution of 4 cm^{-1} on a Perkin-Elmer 1725 X FTIR spectrometer equipped with an automatic data acquisition system. The sample pellets used were prepared by pressing a mixture of 1 mg of well-ground dry HA sample and 60 mg of infrared-grade KBr.

The ^{13}C NMR spectra of the eight HA fractions were recorded on a Bruker DRX-400 NMR spectrometer (Billerica, MA) operated at a ^{13}C frequency of 100.63 MHz and at a magic-angle-spinning (MAS) rate of 6.0 kHz. The solid HA samples were filled in a 4-mm diameter ZrO_2 rotor with a Kel-F cap. A 1.2-s recycle time and a 1-ms contact time were used. Each spectrum consisted of 2400 data points and the chemical shifts were referenced externally to glycine (176.03 ppm).

High performance size-exclusion chromatography was conducted on an HPLC (HP1100 series) equipped with a diode array detector following the method of Zhou et al. (2000). The column (300 $\times$ 7.8 mm) and the guard column (30 by 7.8 mm) used were Biosep-Sec-S2000 (Phenomenex, Torrance, CA). The eluent used was 0.1 M NaCl solution buffered at pH 6.8 with 2 mM phosphate solution and the flow rate was 1.0 mL min^{-1}. The molecular weight calibration was performed with sodium polystyrene sulfonates (PSS, Scientific Polymer Inc.,Warrington, PA) with the molecular weight of 5, 8, 15, 35, and 60 kDa. Blue dextra (2000 kDa, Sigma, St. Louis, MO) and acetone (58 Da, HPLC grade, Fisher, Springfield, NJ) were used as probes for void volume (V_0) and total volume (V_t), which accounted for 5.72 and 12.35 mL, respectively. The standards and HA samples were dissolved in the HPSEC mobile phase at a concentration of 100 mg L^{-1}. The detection wavelength was 224 nm for standards and 254 nm for HA fractions. Weight-averaged apparent molecular weights for the humic substances were determined with equations given by Yau et al. (1979). The term "apparent" is used here because the M_w measured for HA macromolecules using this technique may differ variously from their actual M_w because of the effect of solution chemistry and the difference in the configuration and chemistry between HA and the PSS standards.

RESULTS AND DISCUSSION

Elemental Composition

The mass distribution of the eight HA fractions, the elemental compositions, ash contents, and carboxylic and phenolic contents of each fraction are summarized in Table 1. The first three fractions consist of 84.2% of

Table 1. Yield, elemental composition, and contents of functional groups.

	Yield	C†	H†	N†	O†	H/C	O/C	Ash†	COOH§	Ph-OH§
				wt%					wt %	mol kg^{-1}
Fr1	29.0	54.1	4.8	3.7	37.5	1.1	0.52	3.2	4.0	2.8
Fr2	33.4	56.2	4.7	3.9	36.4	1.0	0.49	2.2	3.8	2.5
Fr3	21.8	56.9	5.4	4.4	34.9	1.1	0.46	1.3	3.6	2.4
Fr4	8.1	57.5	6.1	4.0	29.8	1.3	0.39	0.9	3.6	2.1
Fr5	3.3	57.6	6.6	3.9	29.5	1.4	0.38	1.0	3.4	2.0
Fr6	1.9	59.7	7.2	4.0	28.5	1.4	0.36	0.5	2.9	2.0
Fr7	1.4	59.7	7.9	4.2	28.0	1.6	0.35	ND‡	2.5	1.9
Fr8	1.2	60.1	7.6	4.2	28.9	1.5	0.36	ND‡	2.6	1.9

† on an ash-free basis.
‡ not detected.
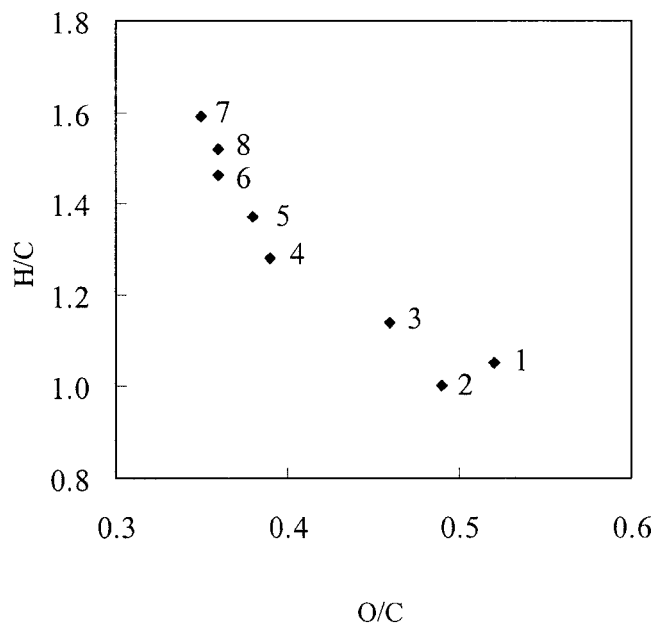
Fig. 1. Plot of H/C vs. O/C atomic ratios for the eight humic acid fractions.

Fig. 2. Fourier transform infrared spectra of the eight humic acid fractions.

the total extracted HAs and the first four fractions consist of 92.3%, indicating that the majority of the HAs can be extracted in the first four cycles. The total mass of the eight fractions represents approximately half of the mass of the original peat soil.

The most important feature of the results in Table 1 is the apparent chemical heterogeneity of the eight HA fractions. The contents of C and H increase from 54.1 and 4.8% of Fr1 to 60.1 and 7.6% of Fr8, respectively, whereas the O content decreases from 37.5 to 28.9%. The N contents remain fairly constant in all fractions. The elemental compositions of Fr1 through Fr3 compare favorably with those of HAs extracted from soils and peats, whereas the elemental compositions of Fr4 through Fr8 have significantly higher contents of H and C and lower contents of O (Mathur and Farnham, 1985; Stevenson, 1994). This indicates that the last five HA fractions, consisting of 16 wt% of the total base-extractable humic acids, are chemically different from the first three HA fractions. The changes of chemical compositions of the eight fractions are well depicted in an H/C vs. O/C atomic ratio diagram (Fig. 1), which is often applied to illustrate the changes in elemental compositions for natural organic materials. Figure 1 shows that the H/C atomic ratio increases gradually from 1.1 for Fr1 to 1.5 for Fr8 and the O/C atomic ratio decreases from 0.52 for Fr1 to 0.36 for Fr8, suggesting that the HAs become more aliphatic and less polar.

The carboxylic and phenolic contents determined by titration decrease from 4.0 and 2.8 mol kg^{-1} for Fr1 to 2.6 and 1.9 mol kg^{-1} for Fr8, respectively (Table 1). Such changes are consistent with the decreasing O/C atomic ratio for the HAs from Fr1 to Fr8.

Infrared Spectra

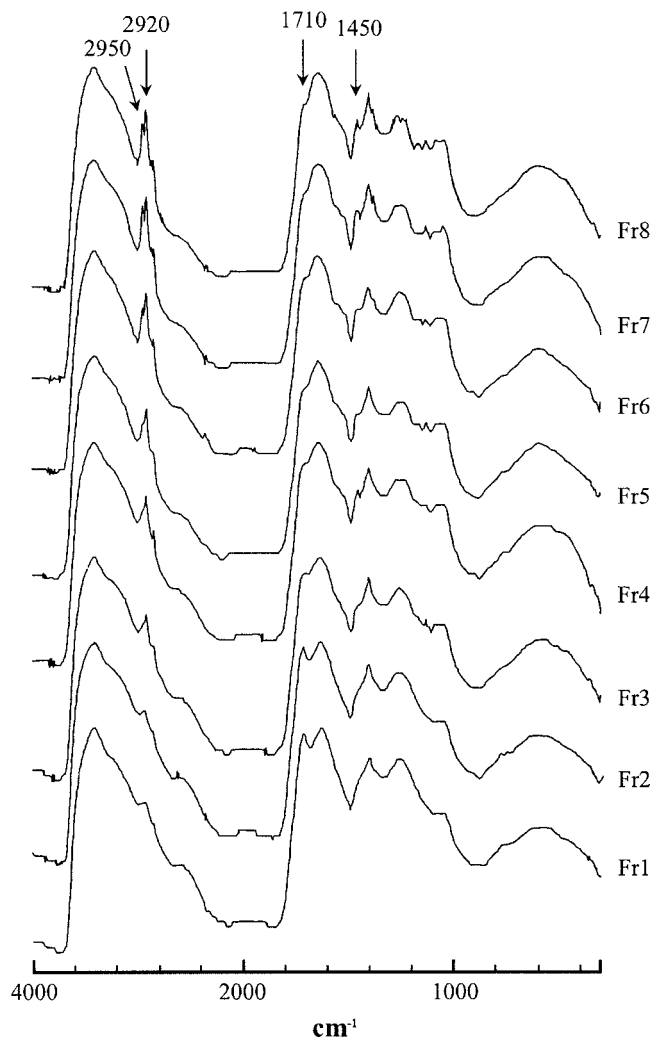Figure 2 presents the FTIR spectra for all eight fractions. The eight fractions exhibit similar IR spectro-

scopic features; that is, a strong H-bonded–OH broad absorption at 3500 to 2500 cm^{-1}, aliphatic C-H stretching absorption from 3000 to 2800 cm^{-1}, carboxyl vibration at 1710 cm^{-1}, conjugated C=C or H-bonded C=O of carbonyl at 1630 cm^{-1} and bending vibration of aliphatic C–H at 1450 cm^{-1} (Stevenson, 1994). Inspection of the spectra reveals that the intensity of 2920 and 2950 cm^{-1} (stretching absorption of –CH$_3$ and –CH$_2$–) becomes progressively more pronounced, and so does the distorted vibration of the two groups at 1450 cm^{-1} but to a lesser extent, indicating an increase of aliphatic C content in HA molecular structures. Conversely, the carboxyl absorption at 1710 cm^{-1} gradually decreases from Fr1 to Fr8, suggesting that the fractions extracted early have higher contents of carboxyl groups than the ones extracted later. These observations are consistent with the variations in the elemental compositions and the contents of carboxylic and phenolic functional groups among the eight fractions.

Carbon-13-Nuclear Magnetic Resonance Spectra

The ^{13}C NMR spectra of the eight HA fractions are shown in Fig. 3 and detailed peak assignments and the
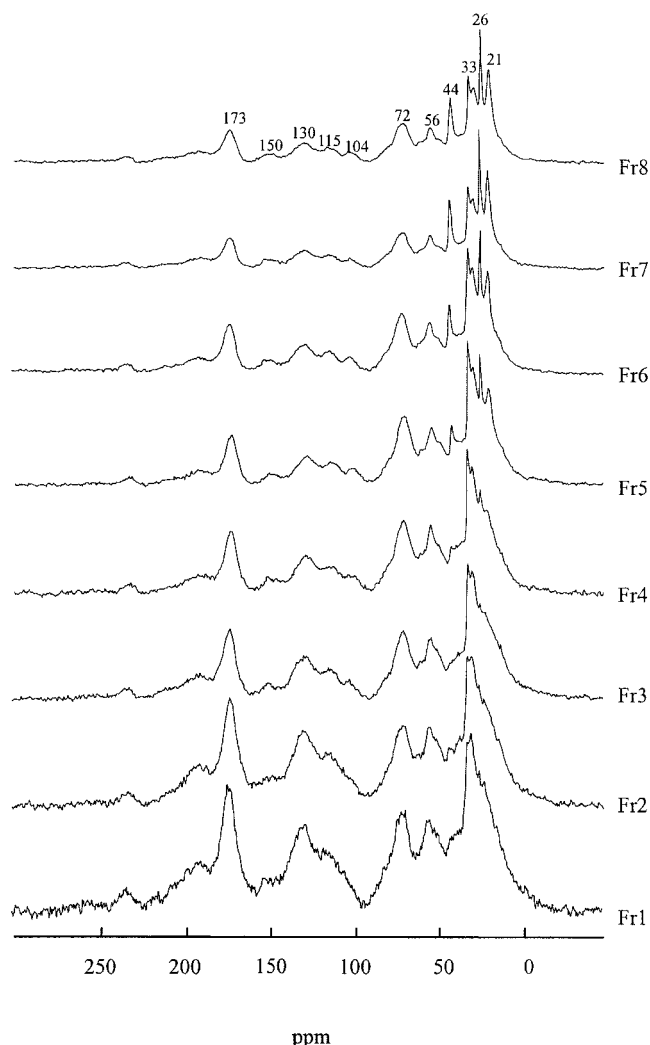
Fig. 3. Cross-polarization magic angle spinning (CPMAS) ^{13}C-NMR spectra for the eight humic acid fractions.

estimated relative percentages of the peak areas are presented in Table 2. In general, the spectra can be divided into four regions, aliphatic C (0–46 ppm), oxygenated aliphatic C (46–108 ppm), aromatic C (108–160 ppm), and carboxylic C (160–185 ppm) (Stevenson, 1994; Preston, 1996; Cook and Langford, 1998).

It is apparent from Fig. 3 that the pattern of the ^{13}C-NMR spectra changes gradually from Fr1 to Fr8, exhibiting significant heterogeneity of molecular structures and functionality among the eight HA fractions. In the aliphatic carbon region (0–46 ppm), the intensities of the peaks increase and several peaks become resolved progressively from Fr1 to Fr8, indicating that the content

of aliphatic carbons increases and the types of the carbons become more diverse as the number of base extraction cycle increases. Fraction 1 has one convoluted peak with a dominant peak identifiable at 30 ppm that likely represents amorphous –(CH$_2$)$_n$– (Hu et al., 2000; Mao et al., 2001). From Fr2 to Fr4 four peaks at 26, 30, 33, and 44 ppm become progressively distinguishable and in Fr5 through Fr8 five peaks at 21, 26, 30, 33 and 44 ppm are sharp and well resolved. According to the literature, these five peaks can be assigned as –CH$_3$ (21 ppm), –CH$_2$– (26 ppm), amorphous –(CH$_2$)$_n$– (30 ppm), crystalline –(CH$_2$)$_n$– (33 ppm), and branched aliphatic carbons (44 ppm)(Wilson, 1987; Schnitzer, 1991; Hu et al., 2000; Mao et al., 2001).

Different HA fractions also have different contents of the five aliphatic carbons as indicated by different intensities of the peaks. The intensities of the CH$_3$ peak at 21 ppm, CH$_2$ peak at 26 ppm, and branched aliphatic carbon peak at 44 ppm increase from Fr1 to Fr8 and the CH$_2$ peak becomes the strongest peak in Fr 6 through Fr8. The intensity of the crystalline aliphatic carbons at 33 ppm appears to increase from Fr1 to Fr3 at the expense of the amorphous aliphatic carbons at 30 ppm. The intensity remains constant but consistently higher than the amorphous aliphatic carbons in Fr4 to Fr8.

The crystalline or rigid vs. amorphous or mobile aliphatic carbons were assigned respectively for peaks at 33 and 30 ppm based on similar ^{13}C-NMR studies of HAs (Kögel-Knabner et al., 1992; Hu et al., 2000; Mao et al., 2001). Kögel-Knabner et al. (1992) distinguished these two types of aliphatic carbons in the neighborhood of 30 ppm and found that the content of the rigid aliphatic carbons relative to the amorphous aliphatic carbons increases with soil sampling depth. It is believed that, compared with the amorphous aliphatic carbons, the crystalline –(CH$_2$)$_n$– chains are relatively more resistant to environmental changes and chemically inert and thus have longer residence times in soil (Hu et al., 2000). The existence of possible crystalline aliphatic carbons in Fr4 through Fr8 is also suggested by the greater resolution of multiple resonance peaks of these HA fractions. It is known that a random mixture of various paraffinic carbons exhibits a broad, unresolved peak such as that of Fr1and Fr2, whereas a paraffinic carbon mixture having finite molecular configurations exhibits sharp and well-resolved peaks such as those present in the latter fractions (Hatcher et al., 1981).

In the oxygenated aliphatic carbon region (46–108 ppm), three peaks at 56, 72, and 104 ppm can easily be identified for all the HA fractions, and their relative intensities appear to be constant in all eight HA frac-

Table 2. Structural group analysis of solid state CPMAS ^{13}C-NMR spectra (%).

	185–160	160–108	108–92	92–64	64–46	46–0	Aromaticity
Fr1	17.5	29.1	4.1	6.9	10.0	32.4	35.3
Fr2	15.6	30.7	3.1	7.6	10.0	32.9	36.4
Fr3	13.8	24.8	1.9	9.2	11.5	38.9	28.8
Fr4	12.1	21.5	2.4	11.5	11.5	41.0	24.5
Fr5	10.7	19.2	2.3	11.7	12.1	44.1	21.5
Fr6	11.2	20.1	2.5	11.0	11.6	43.6	22.6
Fr7	10.8	19.7	2.0	9.2	10.3	48.1	22.1
Fr8	10.8	19.4	2.4	9.8	10.4	47.2	21.7

tions. They can be assigned to methoxyl groups (50–65 ppm), O-alkyl groups (65–95 ppm) (i.e., –CH(OH)– or –CH$_2$–O–C–), and dioxygenated-alkyl groups (95–108 ppm) (i.e., acetal and ketal) (Wilson, 1987; Mao et al., 2001). The latter peak also may be because of unprotonated aromatic carbons (Preston et al., 1997). It is believed that methoxyl groups originate mainly from lignin, and the latter two groups from carbohydrate.

Three peaks are identified in the aromatic carbon region (108–160 ppm) at 115, 130, and 150 ppm, because of protonated, substituted and O-substituted aryl carbons, respectively. The ratio of the peak area integrated over 108 to 160 ppm to that over 0 to 160 ppm is a measure of the aromaticity of an HA, which decreases generally from 35.3% for Fr1 to 21.7% for Fr8. This indicates that the content of aliphatic carbon increases from Fr1 to Fr8, consistent with elemental analyses.

A distinct peak appears in the 160 to 185 ppm range in all eight HA fractions, which is commonly assigned as carboxylic carbons including free carboxylic acids and esters with possible contributions from peptides and quinones (Amalfitano et al., 1995). The intensity of this peak gradually decreases from Fr1 to Fr8, corresponding to the decreasing content of carboxylic groups. This is consistent with the carboxylic acid determination by titration and the gradually reduced absorption intensity at 1710 cm^{-1} in Fourier transform IR (FTIR) spectra.

Distribution of Apparent Molecular Weights

The chromatograms obtained from HPSEC experiments are shown in Fig. 4 and the calculated average values of the M$_w$ are listed in Table 3. It is clear from Fig. 4 that there are two peaks in the chromatogram of Fr1, likely representing two subunits or groups of HAs having average M$_w$ below 10 kDa and above 30 kDa, respectively. The peak area of the HA subunit having smaller M$_w$ is much greater than that of the subunit of larger M$_w$. Fraction Fr2 and Fr3 exhibit a similar pattern of size distribution, but the peak areas of the large-size HA subunit increase appreciably. From Fr4 to Fr8 the large-size HA subunit exhibits a sharp peak with high intensity on each chromatogram and it appears to be a dominant component of each bulk HA fraction. Conversely, the chromatogram in the region of M$_w$ <15 kDa manifests a broad but weak peak, indicating that the small-size HA subunit becomes less abundant.

To better illustrate the heterogeneity of HA molecules among the eight HA fractions, the relative peak area of the two subunits was calculated for each fraction. The results listed in Table 3 indicate that the small-size
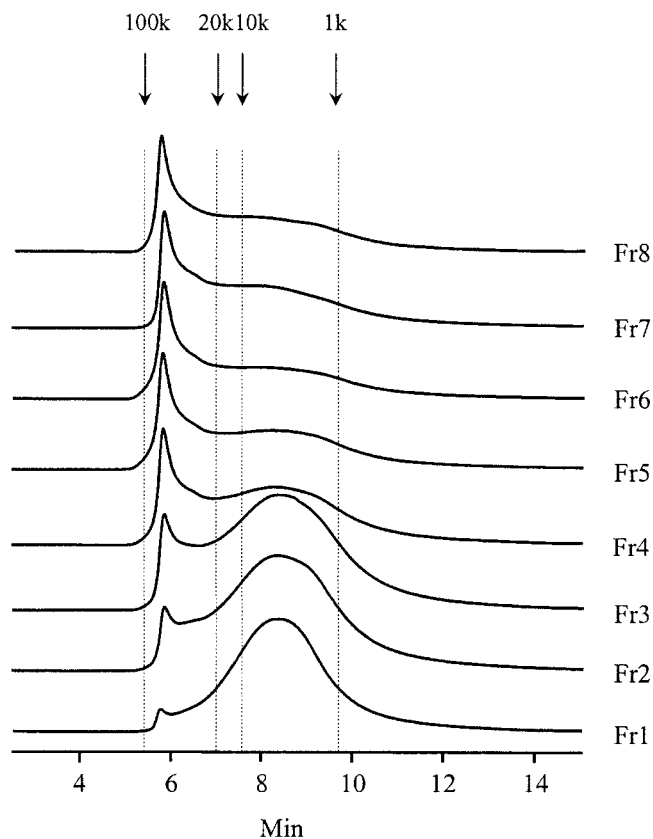

Fig. 4. Chromatograms of high performance size exclusion chromatography for the eight humic acid fractions.

HA subunit has an average M$_w$ of 4.3 to 5.9 kDa and that its peak area decreases from 91% for Fr1 to approximately 60% for Fr5 through Fr8. The large-size HA subunit has an average M$_w$ of 39 to 50 kDa and its relative peak area increases from Fr1 to Fr8 accordingly. Such changes cause a dramatic increase in the average apparent M$_w$ from 7.7 kDa for Fr1 to above 20 kDa for Fr5 through Fr8 (Table 3).

One important feature of the results shown in Tables 1 through 3 is that the variations in M$_w$ among the eight HA fractions appear to correlate with the heterogeneity of chemical compositions and functionality. Figure 5 shows that the H/C atomic ratio decreases and the O/C atomic ratio increases as a function of the peak area of the small-size HA subunit. Regression of the atomic ratios against the peak area of the small-size HA subunit results in two linear correlations with R^2 values >0.84. Figure 6 indicates that the carboxylic and phenolic contents determined chemically increase as a function of

Table 3. Distribution of average apparent molecular weights (kDa).

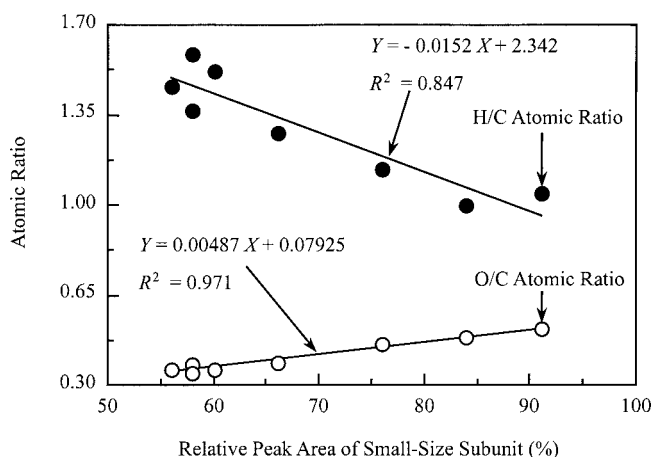| | Average M$_w$ | Large-size HA | | Small-size HA | |
		Average M$_w$	Peak Area%	Average M$_w$	Peak Area%
Fr1	7.7	38.5	9	4.3	91
Fr2	10.6	40.5	16	4.8	84
Fr3	14.4	44.0	24	5.0	76
Fr4	19.6	48.0	33	5.2	66
Fr5	24.2	50.5	42	5.5	58
Fr6	25.4	50.0	44	5.5	56
Fr7	23.2	46.0	42	5.9	58
Fr8	22.1	46.0	40	5.5	60

Fig. 5. Changes of H/C and O/C atomic ratios as a function of the peak area of the small-size humic acid (HA) subunit for the eight HA fractions. Symbols represent data points and lines are the best fits using linear regressions.

Fig. 7. Changes of aromaticity as a function of the peak area of the small-size humic acid (HA) subunit for the eight HA fractions. Symbols represent data points and solid lines are the best fits using linear regressions.

the peak area of the small-size HA subunit. The results of linear regression indicate that the phenolic content appears to correlate well with the peak area of small-size subunit and so do the carboxylic content if the three data points of Fr6 through Fr8 are removed. Figure 7 shows a nearly linear decrease of aromaticity from Fr1 to Fr8 as a function of the peak area of the small-size HA subunit.

The linear correlation between the chemical, molecular, and functional properties of the eight HA fractions in Fig. 5 through 7 indicate that the small-size HA subunit is more aromatic and polar in nature and has higher contents of carboxylic and phenolic functional groups whereas the large-size HA subunit is more aliphatic and less polar in nature. The small-size subunit appears more soluble in base solution than the large-size subunit, resulting in larger quantity of HA in early extraction batches. Extrapolation of the fitted equations listed in Fig. 5 through 7 toward the right sides of the plots yields an H/C atomic ratio of 0.82, an O/C atomic ratio of 0.57, and a phenolic content of 2.9 mol kg^{-1} for the

small-size HA subunit. Its carboxylic content extrapolated from the dotted fitting line in Fig. 6 is approximately 4.4 mol kg^{-1}. Such elemental and functional compositions are typical for humic acids extracted from soils (Stevenson, 1994). Similarly, extrapolation of the fitted equations to 0% of the axis of Fig. 5 yields an H/C atomic ratio above 2 and an O/C atomic ratio below 0.1 for the large-size HA subunit, suggesting that the large-size HA subunit might be fatty acid-like material.

The differences of chemical and molecular properties between the two different HA subunits may result from their differences in source materials. It is likely that the aliphatic subunit was derived biogeochemically from lipid-rich algal or membranes of plant cell wall (Goodwin and Mercer, 1983; Stevenson, 1994) whereas the aromatic subunit was derived from lignin or plant tissue materials. It is known that anoxic conditions of peatland favor preservation of lipids (Stevenson, 1994). Incorporating fatty acid fragments in HA macromolecules is very likely. The measured large M_w of the aliphatic subunit may be indicator of their linear structures under moderate concentration of the background electrolytes. Their relatively hydrophobic composition may cause larger cage structures in aqueous solutions.

This study demonstrates that the difference in base solubility between the two subunits of HA can generate a spectrum of HA fractions that have highly heterogeneous properties. It is likely that the aliphatic HA subunit is less soluble in base solution and hence requires greater solution/soil ratio to be completely extracted, whereas the aromatic HA subunit is very soluble in base solution, is easily extracted, and its relative mass proportion decreases rapidly as a function of extraction. Such differences in base extractability between the two subunits results in fractionation of the HA during the repetitive base extraction of the peat sample and generates large variations in elemental compositions, contents of functional groups and different structure units among the eight fractions because of mixing of the two subunits at different ratios as shown in Fig. 5 through 7.

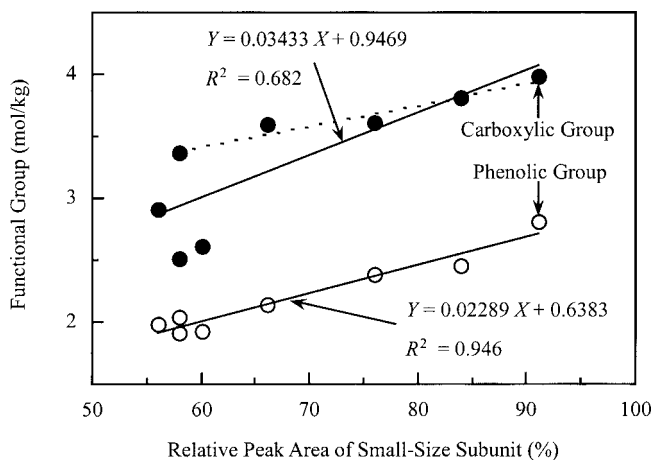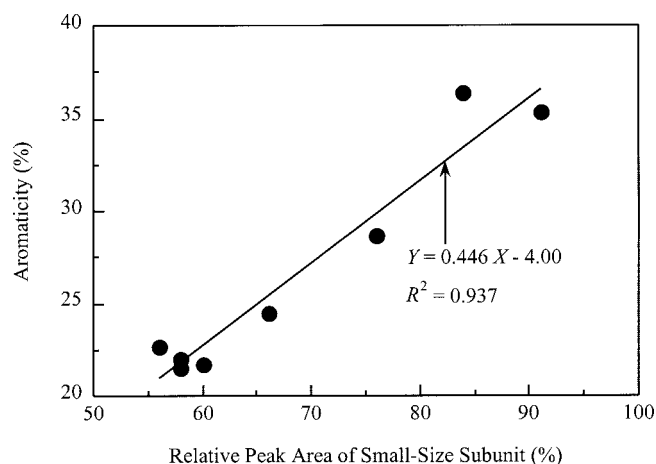Although it is a minor component compared with the

Fig. 6. Changes of carboxylic and phenolic contents as a function of the peak area of the small-size humic acid (HA) subunit for the eight HA fractions. Symbols represent data points and solid lines are the best fits using linear regressions. The dotted line represents the linear fitting of the data excluding Fr6 through Fr8.

aromatic subunit, the aliphatic HA subunit is expected to have much greater impacts on binding of less polar or nonpolar organic pollutants because of its relatively hydrophobic nature. A recent study by Salloum et al. (2002) indicates that the organic C normalized-capacity parameter (K_{OC}) measured for sorption of phenanthrene by naturally occurring organic matter, including HA, correlates well with the contents of parafinic C. Accurate quantification and characterization of this fraction can greatly facilitate elucidation of the mechanisms and enhance predictability of HA-pollutant interactions.

ACKNOWLEDGMENTS

REFERENCES

Amalfitano, C., R.A. Quezada, M.A. Wilson, and J.V. Hanna. 1995. Structure of humic acids: A comparison with precursor litter from different vegetations using spectroscopic techniques. Soil Sci. 159: 391–401.

Barak, P., and Y. Chen. 1992. Equivalent radii of humic macromolecules from acid-base titration. Soil Sci. 152:184–195.

Bartschat, B.M., S.E. Cabaniss, and F.M.M. Morel. 1992. Oligoelectrolyte model for cation binding by humic substances. Environ. Sci. Technol. 26:284–294.

Chiou, C.T., D.E. Kile, D.W. Rutherford, G. Sheng, and S.A. Boyd. 2000. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: Potential sources of the sorption nonlinearity. Environ. Sci. Technol. 34:1254–1258.

Cook, R.L., and C.H. Langford. 1998. Structural characterization of a fulvic acid and a humic acid using solid-state ramp-CP-MAS ^{13}C nuclear magnetic resonance. Environ. Sci. Technol. 32:719–725.

Goodwin, T.W., and E.I. Mercer. 1983. Introduction to Plant Biochemistry. 2nd ed. Pergamon, Oxford.

Hatcher, P.G., G.E. Maciel, and L.W. Dennis. 1981. Aliphatic structure of humic acids: A clue to their origin. Org. Geochem 3:43–48.

Hu, W.-G., J.-D. Mao, B. Xing, and K. Schmidt-Rohr. 2000. Poly (methylene) crystallites in humic substances detected by nuclear magnetic resonance. Environ. Sci. Technol. 34:530–534.

Kögel-Knabner, I., P.G. Hatcher, E.W. Tegelaar, and J.W. de Leeuw. 1992. Aliphatic components of forest soil organic matter as determined by solid-state ^{13}C NMR and analytical pyrolysis. Sci. Total Environ. 113:89–106.

Lyon, W.G. 1995. Swelling of peats in liquid methyl, tetramethylene and propyl sulfoxides and in liquid propyl sulfone. Environ. Toxicol. Chem. 14:229–236.

Mao, J.-D., W.-G. Hu, K. Schmidt-Rohr, G. Davies, E.A. Ghabbour, and B. Xing. 2000. Quantitative characterization of humic substances by solid-state carbon–13 nuclear magnetic resonance. Soil Sci. Soc. Am. J. 64:873–884.

Mao, J.-D., B. Xing, and K. Schmidt-Rohr. 2001. New structural information on a humic acid from two-dimensional ^{1}H-^{13}C correlation solid-state nuclear magnetic resonance. Environ. Sci. Technol. 35: 1928–1934.

Mathur, S.P., and R.S. Farnham. 1985. Geochemistry of Humic Substances in Natural and Cultivated Peatlands. p. 53–85. *In* G.R. Aiken et al. (ed.) Humic Substances in Soil, Sediment, and Water. John Wiley & Sons, New York.

Piccolo, A., P. Conte, E. Trivellone, B. van Lagen, and P. Buurman. 2002. Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and ^{1}H-NMR spectroscopy. Environ. Sci. Technol. 36:76–84.

Preston, C.M. 1996. Application of NMR to soil organic matter analysis: History and prospects. Soil Sci. 161:144–166.

Preston, C.M., J.A. Trofymow, B.G. Sayer, and J. Niu. 1997. ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to access litter quality in decomposition studies. Can. J. Bot. 75:1601–1613.

Salloum, M.J., B. Chefetz, and P.G. Hatcher. 2002. Phenanthrene sorption by aliphatic-rich natural organic matter. Environ. Sci. Technol. 36:1953–1985.

Schnitzer, M. 1991. Soil organic matter-the next 75 years. Soil Sci. 151:41–58.

Stevenson, F.J. 1994. Humus chemistry: Genesis, composition, reactions. 2nd ed. John Wiley & Sons, New York.

Swift, R.S. 1996. Organic matter characterization. p. 1011–1069. *In* D.L. Sparks et al. (ed.) Methods of soil analysis. Part 3. SSSA Book Series No.5. SSSA and ASA, Madison, WI.

Weber, J.H. 1988. Binding and transport of metals by humic materials. p. 165–178. *In* F.H. Frimmel and R.F. Christman (ed.) Humic substances and their role in the environment. John Wiley & Sons, Chichester, UK.

Wilson, M.A. 1987. NMR techniques and applications in geochemistry and soil chemistry. Pergamon, Oxford, UK.

Xia, G., and J.J. Pignatello. 2001. Detailed sorption isotherms of polar and apolar compounds in a high organic soil. Environ. Sci. Technol. 35:84–94.

Xing, B., and J.J. Pignatello. 1997. Dual-mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter. Environ. Sci. Technol. 31:792–799.

Yau, W.W., J.J. Kirkland, and D.D. Bly. 1979. Modern size exclusion chromatography. John Wiley & Sons, New York.

Zhou, Q.H., S.E. Cabaniss, and P.A. Maurice. 2000. Considerations in the use of high-pressure size exclusion chromatography (HPSEC) for determining molecular weights of aquatic humic substances. Water Res. 34:3505–3514.