SHORT COMMUNICATIONS

Estimating the Percent Aromatic Carbon in Soil and Aquatic Humic Substances Using Ultraviolet Absorbance Spectrometry

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ABSTRACT

The aromatic C content of humic substances is an important chemical property that can be used to explain formation, source, and potential interactions of the humic substances with pesticides and other contaminant organics. The relationship between the UV absorptivity at 272, 254, and 205 nm of six aquatic fulvic acids, eight soil fulvic acids, and four water soluble organic C (WSOC) fractions, and their aromatic C content, as determined by ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (CPMAS NMR) spectroscopy was evaluated. The percent aromatic C (determined by NMR) for the pooled soil and aquatic fulvic acids and WSOC fractions data was poorly correlated ($r < 0.7$) with the UV absorptivity measurements at all three wavelengths. When the soil fulvic acid was considered individually, however, a relatively high correlation ($r = 0.80$ to 0.85, $P < 0.05$) resulted for the prediction of aromatic C content using UV absorptivity. Poor correlations were found for the other individual fractions. Our data suggest that the prediction of aromatic C content using UV absorptivity was applicable only for base-extracted soil fulvic acids and not for the aquatic fulvic acids and soil WSOC fractions used in this study.

ESTIMATION of the aromatic C content of humic substances provides information that may be used to explain their formation (Stevenson, 1982), provide clues as to their origin (Hatcher et al., 1980), and for evaluation of potential interactions with pesticides and other organic contaminants (Gauthier et al., 1987). Currently, ^{13}C cross polarization, magic angle spinning nuclear magnetic resonance (CPMAS NMR) spectroscopy represents the "state-of-the-art" instrumentation available for estimating the aromatic C content of humic substances (Malcolm, 1989; Wilson, 1989). Unfortunately, both the limited availability and the high expense of NMR spectrometers and measurements preclude the use of this method for routine characterization studies. Clearly, a more convenient method of ascertaining the aromatic C content of humic substances is desirable. We are conducting studies investigating the migration of fulvic acids and water soluble organic C (WSOC) from Coastal Plain soil to blackwater streams and then evaluating the potential interaction of these fractions with nonionic organic contaminants. We needed a rapid and semiquantitative method for the determination of the aromatic C content of these two fractions, and therefore, decided to evaluate the ultraviolet (UV) absorbance method proposed by Traina et al. (1990). They reported a high correlation ($r = 0.94$) between the absorptivity of humic acids at 272 nm and the aromatic C content as measured by ^{13}C NMR spectroscopy. The application and utility of this method has not been evaluated on a large range of humic substances including fulvic acids or WSOC extracts. The purpose of our investigation was to evaluate the utility of the UV absorbance method for rapid, routine estimates of aromatic C contents by comparing the UV absorptivity of six aquatic fulvic acids, eight soil fulvic acids, and four soil WSOC extracts, measured at 272, 254, and 205 nm, to the percent aromatic C content as determined by ^{13}C CPMAS NMR spectroscopy.

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MATERIALS AND METHODS

Isolation and Extraction of Humic Substances

The organic substances used in this study were isolated from eight South Carolina soils, four Ohio soils, and two Coastal Plain blackwater streams in South Carolina.

Soil Fulvic Acids. Soil fulvic acids SF-1 to SF-4 (Tables 1 and 2) were obtained from a previous study (Novak and Smeck, 1991). These fulvic acids were alkali-extracted from surface horizons of four Ohio Glacial Till soils, and the C contents and structural properties (as revealed by ^{13}C CPMAS NMR) were determined using methods outlined in Novak and Smeck (1991). The taxonomic and chemical characteristics of the soils and fulvic acids are provided in Tables 1 and 2.

Soil fulvic acids SF-5 to SF-8 (Tables 1 and 2) were

Table 1. Taxonomic and chemical properties of soils.

Soil	Symbol	Soil taxonomy (subgroup)	pH†	Organic C g kg^{-1} soil
Xenia	SF-1	Aquic Hapludalf	5.9	14.8
Dana 2‡	SF-2	Typic Hapludoll	6.6	21.0
Rossmoyne	SF-3	Aquic Fragiudalf	7.5	11.0
Dana 1	SF-4	Typic Hapludoll	5.8	24.9
Lucy	SF-5	Arenic Paleudult	5.7	15.5
Troup	SF-6	Grossarenic Paleudult	5.7	36.5
Pickney	SF-7	Cumulic Humaquept	5.3	32.9
Pickney	SF-8	Cumulic Humaquept	4.8	30.5
Troup	WSOC-1	Grossarenic Paleudult	4.8	9.8
Pickney	WSOC-2	Cumulic Humaquept	5.3	32.9
Osier	WSOC-3	Typic Psammaquent	4.2	86.0
Lucy	WSOC-4	Arenic Paleudult	5.7	15.5

† pH taken at a 2:1 soil/water ratio.
‡ Variant of Dana soil series.

alkali-extracted from surface horizons of four South Carolina Coastal Plain soils using a modified method of Novak and Smeck (1991). The modifications involved conducting the extractions under a Ar atmosphere, and omitting passage of fulvic acid through XAD-8 resin. The fulvic acids were purified by passage through a Bio-Rad (Bio-Rad Labs, Richmond CA) AG50W-X8 cation exchange resin. All fulvic acids were then evaporated to dryness at 40 °C.

Water Soluble Organic Carbon. The WSOC fraction was also extracted from surface horizons of four South Carolina Coastal Plain soils using three sequential Millipore Milli-Q H_2O extractions at a 1:1 soil/water ratio, shaken for 45 min, then centrifuged at 3400 *g*, and the supernatant decanted. The supernatant was filtered through a Millipore Milli-Q H_2O prerinsed Whatman no. 42 filter paper (Whatman Int., Maidston, England), followed immediately by filtering through a prerinsed 0.4-μm polycarbonate Nuclepore filter (Nuclepore Corp., Pleasanton, CA). The WSOC fractions were freeze-dried.

Aquatic Fulvic Acids. The aquatic fulvic acids were extracted from Upper Three Runs Creek near Box Landing (samples AF-1 to AF-3 in Table 2) and Tinker Creek (samples AF-4 to AF-6 in Table 2) during base flow periods in April, October, and November of 1988. Both blackwater streams are low gradient, mildly acidic, low in nutrients, (Lower, 1985) and are located on the Department of Energy's Savannah River site. The topography of the watershed is characterized by rolling upland ridges and wide floodplain areas, which are typical landforms in the Upper Atlantic Coastal Plain region (Siple, 1967).

Large volumes (>500 L) of stream water were filtered through a Whatman glass fiber filter (Whatman GF/C, Whatman Int., Maidston, England), acidified with conc. HCl to a pH of <2, and stored in plastic carboys for 1 to 2 d. The fulvic acids were then extracted using XAD-8 resin (Rhom and Haas, Philadelphia, PA) and purified using Bio-Rad (Bio-Rad Labs, Richmond, CA) AG50W-X8 cation-exchange resin as described by Thurman and Malcolm (1981). The samples were freeze-dried.

Carbon Analysis

The mean C contents of the aquatic fulvic acids, samples SF-5 to SF-8, and all WSOC fractions were determined (in triplicate) using a Perkin-Elmer (Perkin-Elmer Corp., Los Angeles, CA) model 240C analyzer. The results are presented in Table 2 on a dry, ash-free basis.

Carbon-13 CPMAS NMR and UV Spectroscopic Measurements

Soil fulvic acid samples SF-1 to SF-4 (Tables 1 and 2) were analyzed using ^{13}C CPMAS NMR spectroscopy as described in Novak and Smeck (1991). For each spectrum, the percent C in the aromatic C portion was estimated from the chemical shift region between 110 to 160 ppm (Malcolm, 1989) by dropping a vertical line to an arbitrarily drawn baseline and measuring the area.

Table 2. Properties of aquatic fulvic acids (AF), soil fulvic acids (SF), and soil water soluble organic C (WSOC). Carbon contents are reported on a dry, ash-free basis.

Humic sample	C content	Percent + aromatic C	Absorptivity, nm‡ 272	254	205
	mmol g^{-1}	%			
		Aquatic fulvic acids			
AF-1	41.6	13.8	29.2	35.2	72.6
AF-2	38.2	14.2	16.7	18.1	48.6
AF-3	35.0	10.0	21.8	25.6	58.3
AF-4	41.1	17.5	26.7	31.6	67.0
AF-5	41.2	14.4	22.0	26.5	60.7
AF-6	47.3	15.2	26.5	31.1	66.7
		Soil fulvic acids			
SF-1	34.7	19.3	26.5	35.1	71.6
SF-2	33.8	18.8	35.8	47.5	88.5
SF-3	38.3	18.8	32.5	42.7	84.0
SF-4	34.8	19.3	28.6	37.8	74.2
SF-5	34.5	15.0	23.9	29.1	53.1
SF-6	38.4	15.0	23.1	27.6	55.8
SF-7	34.5	4.9	17.4	20.7	39.0
SF-8	35.8	12.9	17.7	20.3	44.7
		Soil water soluble organic C			
WSOC-1	43.3	5.5	32.6	38.3	71.2
WSOC-2	34.2	7.3	13.9	16.5	36.6
WSOC-3	32.0	4.2	15.3	19.5	52.3
WSOC-4	35.0	4.9	16.5	21.2	43.0

+ Determined by ^{13}C CPMAS NMR spectroscopy.
‡ Absorptivity values are listed in units of L mg^{-1} cm^{-1} × 1000.

Soil fulvic acid samples SF-5 to SF-8, all aquatic fulvic acids (Tables 1 and 2) and all WSOC fractions were analyzed using ^{13}C CPMAS NMR spectroscopy by the Nuclear Magnetic Resonance Center at Colorado State University. Spectra were obtained on a home-built spectrometer using a Nicolet 1180 data system and a 293B pulse programmer (General Electric, Los Angeles, CA) operating at 25.3 MHz using hexamethylbenzene as an external standard. Spectra were collected using 50 000 scans, a contact time of 1 ms, and a recycle time of 1 s. The percent C in the aromatic C portion of each spectrum (similar region as above) was estimated by integration and is reported in Table 2.

Stock fulvic and WSOC solutions were prepared for UV absorbance measurements by dissolving 50 mg of fulvic acid or WSOC in 200 mL of 50 mmol L^{-1} NaCl, and then adjusting the pH to 7.0 with 0.1 mol L^{-1} HCl. The stock solutions were gently shaken, covered with Al-foil wrap, and left to equilibrate for 24 h at 25 °C. Aliquots of the stock solutions were then added to 100 mL volumetric flasks, diluted to volume with pH 7.0, 50 mmol L^{-1} NaCl to obtain a 6, 12, 18, 24, and 30 mg L^{-1} fulvic acid or WSOC solution. All solutions were prepared in triplicate. Immediately, the UV absorbance of each sample at a wavelength of 272, 254, and 205 nm was measured using a Shimadzu model UV2-1000, UV-VIS spectrophotometer (Mitsubishi Electronics America, Inc., Terrance, CA) equipped with a 1.0-cm path cell length. These three wavelengths were chosen because they represent regions of π-π^* transitions for arenes and polyenes (Streitwieser and Heathcock, 1976; Pavia et al., 1979; Gauthier et al., 1987), which are known structural constituents of most humic substances. The UV absorptivity of each humic substance at 272, 254, and 205 nm was calculated on a dry, ash-free C basis, and was then correlated with the percent aromatic C content (Fig. 1a –

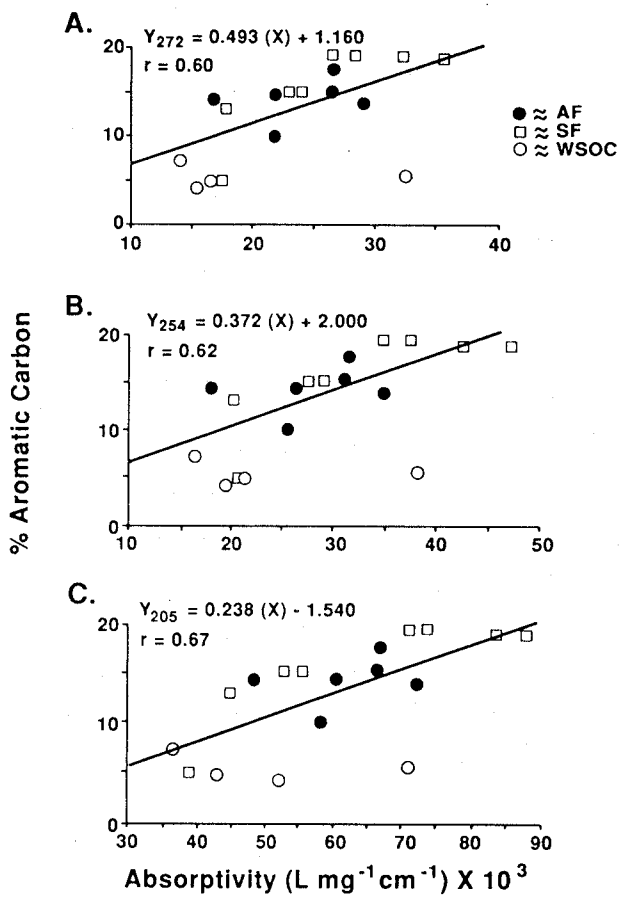

Fig. 1. Relationship between the percent aromatic C of aquatic fulvic acids (AF), soil fulvic acids (SF), and soil water soluble organic C (WSOC) and the UV absorptivity measured at:(*a*) 272, (*b*) 254, and (*c*) 205 nm.

1c) measured by ^{13}C CPMAS NMR spectroscopy. Increases in the aqueous concentrations of fulvic acids and WSOC fractions resulted in a linear increase in light absorbance at wavelengths of 272, 254, and 205 nm. We obtained r values of >0.999 ($P < 0.05$) for each set of humic substance dilutions when correlated with the absorptivity measurements.

RESULTS AND DISCUSSION

The aquatic fulvic acids, soil fulvic acids, and WSOC fractions used in this study were obtained from a variety of sources. The diverse nature of the humic substances resulted in variations in the C and aromatic C contents observed in Table 2.

The r value for the percent aromatic C (as determined by NMR) for the aquatic fulvic acids, soil fulvic acids, and soil WSOC fraction considered as a group was highly significant ($P < 0.01$) but was poorly correlated (0.60 – 0.67) with the UV absorptivity measured at all three wavelengths (Table 3, Fig. 1a, b, and c). When we correlated the UV absorptivity and the aromatic C content of each group of humic substances individually with the absorptivity values at all three wavelengths, a low r value was obtained for the WSOC samples (0.08 – 0.38) and for the aquatic fulvic acids (0.30 – 0.33). In contrast, when only the soil fulvic acids were correlated, we obtained r values

Table 3. Correlation coefficients for the relationship between percent aromatic C content of aquatic fulvic acids (AF), soil fulvic acids (SF), soil water soluble organic C (WSOC) fraction, and the UV absorptivity measured at 272, 254, and 205 nm.

Humic substances	No. of samples	Absorptivity, nm		
		272	254	205
SF	8	0.80*	0.80*	0.85**
AF	6	0.33	0.30	0.31
WSOC	4	0.08	0.15	0.38
WSOC + SF + AF	18	0.60**	0.62**	0.67**

*,**Significant at the 0.05 and 0.01 level of significance, respectively.

of between 0.80 and 0.85 (Table 3), with the highest correlation at 205 nm. The relationship between the aromatic C content and the UV absorptivity at 205 nm for soil fulvic acids was found to be

$$Y = 0.230\,X + 0.826 \qquad [2]$$

where Y is the percent aromatic C, and X is the UV absorptivity in (mL mg^{-1} cm^{-1}). The data indicate that the UV absorptivity measurements at all three wavelengths provided a reasonable estimate of aromatic C in soil fulvic acids, but a poor estimate for all other humic substances examined. The higher r values obtained with the soil fulvic acids may be due to the presence of similar chromophore structures. Failure to obtain a high correlation ($r > 0.8$) using aquatic fulvic acids and the soil WSOC fraction may be due to differences in aromatic substituents, which cause a shift in the absorption wavelength or the presence of other nonaromatic structures containing chromophores, which can absorb UV light at the same wavelengths used in this study.

CONCLUSIONS

The aromatic C content of the suite of aquatic fulvic acids, soil fulvic acids, and WSOC extracts as a group could not be quantitatively estimated using the UV absorbance method at 272, 254, and 205 nm. Working with only the soil fulvic acid fractions, however, we obtained a semiquantitative prediction (r values between 0.80 and 0.85) of the aromatic C content and the UV absorptivity measured at all three wavelengths. On the other hand, the UV absorbance method provided a poor prediction of the aromatic C content for all other humic substances. Therefore, the UV absorbance method for the prediction of aromatic C content proposed by Traina et al. (1990) was applicable for only base-extracted soil fulvic acids and not for aquatic fulvic acids or soil water soluble extracts used in this study.

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