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Title : Water Soluble Organic Fractions in Sewage Sludge Amended Soil : Characterization and Binding Mechanisms with Copper(II)

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V. Van Volk

Application of sewage sludge to soils increases the trace metal content and the organic content of soils. The complexation of indigenous and added metals with organic constituents must be studied to evaluate metal availability to plants and animals.

The water soluble organic fractions (WSOF) from an agricultural soil (W), a soil treated with sludge for 6 years (WS_6), a sludge-soil mixture incubated for 1 week (WS_1), and sewage sludge (SS) were extracted and purified. The WSOF samples were assayed for C, H, N, S, O, P, total acidity, COOH, and phenolic OH, and by UV-visible, infrared, and proton nuclear magnetic resonance spectroscopy. The Cu(II) binding mechanisms in the WSOF samples were studied by electron spin resonance (ESR) spectroscopy and potentiometric titrations. Conditional ratios of complexed metal (Mc) to free metal ions (M) at 296 K were calculated.

The SS contained higher organic H, N, and P contents, a higher H/C, and a lower C/N ratio than W. Total acidity, carboxyl, and phenolic hydroxyl group contents were generally highest in SS, intermediate in WS_6 and WS_1, and lowest in W. Overall aromatic character and aromatic

carboxyl group contents were highest in W, and lowest in SS while the aliphatic proton and carboxyl group contents were highest in SS, and lowest in W. Protein decomposition products were most prevalent in SS, and decreased in concentration as the sludge component in the mixture decreased.

At 77 K, the Cu(II)-W complex showed an anisotropic ESR spectrum while the Cu(II)-SS complex showed an isotropic spectrum. These spectral results suggest that the oxygen donor ligands of W may form relatively strong bonds with Cu(II) due to extensive equatorial chelation while ligands of SS may form little or no chelate bonds with Cu(II).

The ratio of complexed metal to free metal ions in WSOF samples increased as sewage sludge was added to the soil. A maximum Cu(II) binding capacity for W and WS_6 was attained near the value of 0.1 mol Cu(II)/kg WSOF while it was not reached for WS_1 or SS.

Although the bond strength between Cu(II) and W is stronger than the bond between Cu(II) and SS due to chelate bond in the former, the Cu(II) complexing capacity of a soil appears to increase with sludge amendment.

Water Soluble Organic Fractions in Sewage Sludge Amended Soil : Characterization and Binding Mechanisms with Copper(II)

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Water Soluble Organic Fractions in Sewage Sludge Amended Soil : Characterization and Binding Mechanisms with Copper(II)

INTRODUCTION

Sewage sludge is commonly applied to land; however, trace metals in the sludge can accumulate in the plant root zone, and possibly decrease crop yields, contaminate edible plant parts, or both (Silviera and Sommers, 1977). With increased disposal of urban and industrial sewage waste on land, the chemistry of heavy metals in the environment must be further studied (Street et al., 1977).

Since the fulvic acid found in the soil contains a relatively large number of oxygen-containing functional groups, it should figure importantly in the regulation of the trace metal solubility and distribution (Schnitzer and Skinner, 1966). Consequently, fulvic acid extracted by alkali from soil or sewage sludge has been studied extensively (Sposito, 1981). However, little is known about the structural characteristics or coordination chemistry of water soluble organic fractions from soil and sludge materials. Differences are anticipated between fulvic acid extracted with alkali and the water soluble organic fraction extracted with distilled water.

The objectives of this study were:
(i) to investigate changes in the water soluble organic fractions of soils after sewage sludge application, (ii) to further develop a description of the Cu(II) binding mechanisms with water soluble organic materials in sewage sludge amended soil, and (iii) to compare the Cu(II) complexation with water soluble organic materials before and after sewage sludge application.

Characterization of the Water Soluble Organic Fraction Extracted from a Sewage Sludge Amended Soil[1]

Hyungsik Lim, V. V. Volk, and John Baham[2]

ABSTRACT

The water soluble organic fractions (WSOF) from an agricultural soil (W), a soil treated with sludge for 6 years (WS_6), a sludge-soil mixture incubated for one week (WS_1), and sewage sludge (SS) were extracted, purified, and characterized by elemental analysis, functional group determinations, infrared, UV-visible, and proton nuclear magnetic resonance spectroscopy.

The SS was characterized by higher organic H, N, and P contents, a higher H/C ratio, and a lower C/N ratio than W. Total acidity, carboxyl and phenolic hydroxyl group contents were generally highest in SS, intermediate in WS_6 and WS_1, and lowest in W.

Overall aromatic character and aromatic carboxyl group contents were highest in W, and lowest in SS. Aliphatic proton, aliphatic carboxyl, and phenolic hydroxyl group contents were highest in SS, and lowest in W. Protein decomposition products were the pronounced

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components in SS, and decreased in concentration as the sludge component in the mixtures decreased.

The ^{1}H-NMR spectra suggested that the SS-protons were bound to a wider range of functional groups than W-protons. Structural complexities around the aromatic protons followed the following order: SS > WS_1 > WS_6 > W.

Additional Index Words: UV-visible spectroscopy, infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, elemental analysis, functional groups, humic materials.

INTRODUCTION

Sewage sludge is commonly applied to land; however, trace metals in the sludge can accumulate in the plant root zone, and possibly decrease crop yields, contaminate edible plant parts, or both (Silviera and Sommers, 1977). With increased disposal of urban and industrial sewage waste on land, the chemistry of heavy metals in the environment must be further studied (Street et al., 1977).

Since the water-soluble fulvic acid found in the soil solution contains a relatively large number of oxygen-containing functional groups, it should figure importantly in the regulation of the trace metal solubility and distribution (Schnitzer and Skinner, 1966). However, little is known about the structural characteristics or coordination chemistry of water-soluble, metal complexing fractions that exist in sludge-soil mixtures (Holtzclaw et al., 1976).

Fulvic acid is defined as the organic fraction soluble in caustic alkali and in a dilute mineral acid. It is not a single chemical

compound of definite composition, but instead contains a wide range of components of differing composition, functional group acidity, and molecular weight (Schnitzer and Skinner, 1968a).

The methods for extraction and purification of fulvic acid have been discussed extensively (Kononova, 1966; Schnitzer and Khan, 1972; Schnitzer, 1978; Hayes et al., 1975). The extraction techniques have been studied with regard to strength of the alkali extractant (Levesque and Schnitzer, 1966), exposure to air during extraction (Schnitzer and Skinner, 1968b), and the pH to separate the fulvic acid from the humic acid fraction (Sequi et al., 1975).

Methods to purify fulvic acid differ widely. High-speed centrifugation and treatment with a dilute HF-HCl solution have been used to remove clay (Mortensen and Himes, 1964). The HF-HCl reduces the ash content, but also partially destroys the fulvic acid. Inorganic salts usually have been removed by dialysis or cation exchange resins. Low-molecular weight components of fulvic acid, however, may be lost during dialysis (Mortensen and Himes, 1964). The low-molecular weight fulvic acid components have been subsequently recovered by sorption onto activated charcoal and elution either with acetone or with NaOH (Stevenson and Goh, 1971). From elemental and infrared spectral analyses, the low-molecular-weight fulvic components were found to contain significantly more COOH groups than the retained fulvic acid. These COOH components are likely to react with trace metals (Gamble and Schnitzer, 1973). Fulvic acid has been extracted and purified from a sludge-soil mixture without dialysis (Holtzclaw et al., 1976); however, the method involved 0.5M NaOH treatment and subsequent acidification to pH 1.0 with HCl.

Water soluble fulvic acid from soil or sewage sludge has been studied extensively (Sposito, 1981); however, little is known about the water soluble organic fractions from soil and sludge materials. The objective of this study was to investigate changes in the water soluble organic fractions of soils after sewage sludge application.

MATERIALS AND METHODS

Water soluble organic fractions (WSOF) were prepared from (i) Willamette silt loam soil (fine-silty, mixed, mesic, Pachic Ultic Argixeroll), (ii) a soil treated with sludge for 6 years (annual application of 25 kg/m^2 of sewage sludge and 45 g N/m^2 of $(NH_4)_2SO_4$ fertilizer), (iii) a sludge-soil mixture incubated for one week in the laboratory (equivalent to an application rate of 50 kg/m^2 of sewage sludge), and (iv) sewage sludge from Portland, Oregon. All discussion of the soil and sludge characteristics will refer to only the WSOF of each sample.

Air-dried or sludge treated soil (100 g) and 500 mL of distilled water were placed in a 1-liter polyethylene bottle, bubbled with high-purity N_2 gas to displace air, capped, and shaken for 18 hours at 297±2 K. No distilled water was added to the sewage sludge (100 g, 4% solid). The mixture was transferred to 250-mL polyethylene bottles and centrifuged for 20 min at 7970 g. The supernatant solution was decanted and vacuum filtered through a no. 42 Whatman filter paper held on a Büchner funnel under N_2 gas. The filtrate was centrifuged at 27000 g for 20 min in 50-mL polyethylene centrifuge tubes to further remove colloidal-size material. The supernatant was transferred to a 1-liter polyethylene bottle and stored at 253 K.

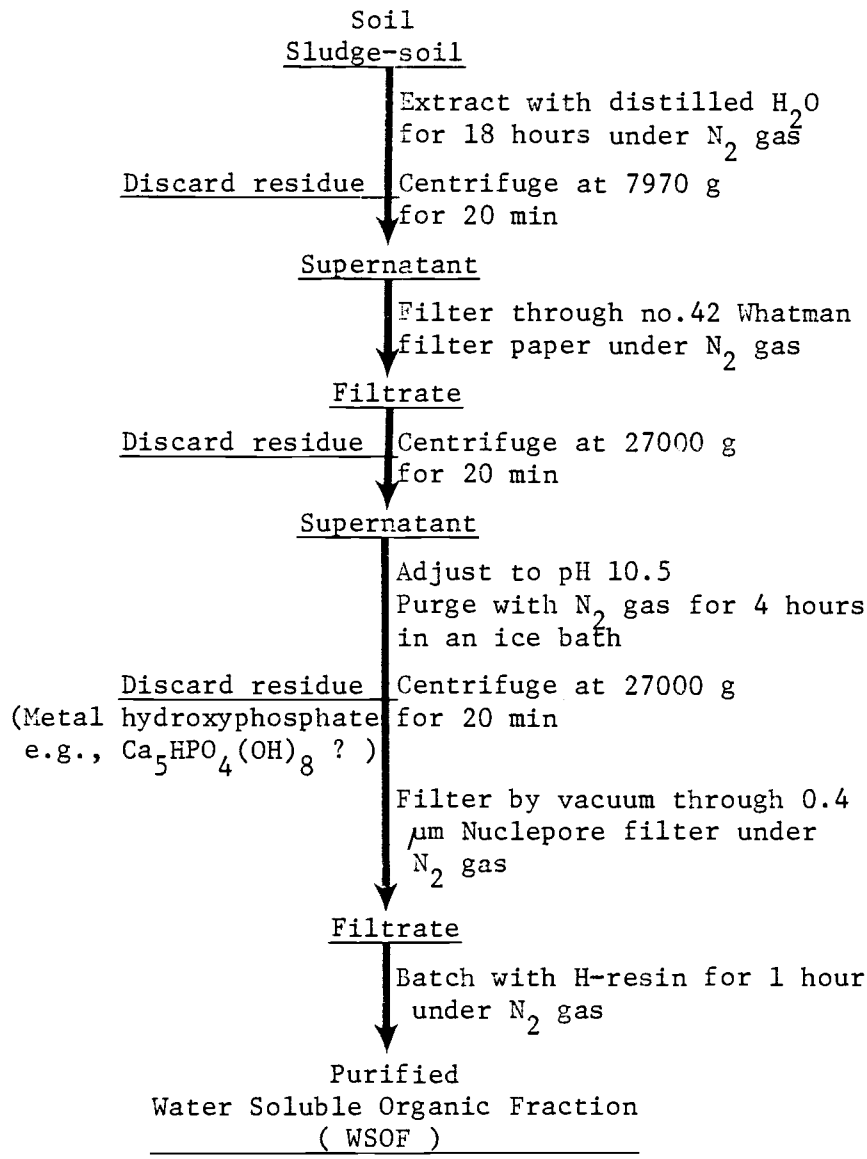

Figure 1. Procedures used to extract and purify water soluble organic fraction.

The WSOF was purified by adjustment of the solutions to pH 10.5 with dilute NaOH. The solution was purged with N_2 gas for 4 hours in an ice bath to remove NH_3 and to precipitate phosphate. The solution was then centrifuged at 27000 g for 20 min and white precipitates were discarded. The supernatant was vacuum filtered through a 0.4 μm Nuclepore filter under N_2 gas to remove the fine clay and particulate organic matter (Malcolm, 1976). The filtrate was then placed in a 2-liter polyethylene beaker and stirred for 1 hour, under N_2 gas, with an analytical grade H^+-saturated cation exchange resin [Rexyn 101(H), 16-50 mesh, medium-porosity] to remove trace metals. The purified water soluble organic fraction was stored at 253 K. When required, the dilute water soluble organic fraction was concentrated to 1 to 4 mg/mL on an ash-free basis using a rotary evaporator at 323 K.

Elemental and Functional Group Analysis

The purified water soluble organic fractions (WSOF) were analyzed for total C by wet combustion using a Beckman Carbon Analyzer. Hydrogen was determined by the standard Pregl dry combustion procedure (Niederl and Niederl, 1942). Total N was determined by the conventional semimicro-Kjeldahl method, with the WSOF sample digested in sulfuric-salicylic acid solution (Bremner, 1965). Phosphorus was determined colorimetrically (Berg and Gardner, 1978) after perchloric acid digestion. Sulfur was determined gravimetrically by precipitation as $BaSO_4$ after oxidation of the sample (Sposito et al., 1976). The oxygen in the sample was calculated as the difference between 100 and the sum of C, H, N, and S expressed on a water and ash-free basis. Metal concentrations were measured by atomic absorption spectrophoto-

metry. Chloride, nitrate, and ammonium in the samples were determined with the appropriate ion selective electrode (ISE).

Total acidity was determined by the $Ba(OH)_2$ method (Schnitzer and Khan, 1972). Carboxyl groups were determined by the calcium acetate method modified by steam distilling the WSOF-calcium acetate solution and titrating the distillate to pH 8.50 with standard base (Holtzclaw and Sposito, 1979). Ash contents of the samples were determined by ignition (Holtzclaw et al., 1976).

Spectroscopic Measurements

UV-visible spectra

The UV-visible spectra of WSOF solutions (0.05 and 0.5 g L^{-1} WSOF in 0.05 M $NaHCO_3$ for UV and visible spectra, respectively) were obtained on a Varian CARY 219 double beam spectrophotometer. Spectra were recorded from 700 to 200 nm at about 296 K using a scan rate of 50 nm/min. The ratio E_4/E_6 was determined by measurement of the absorbance of each WSOF solution at 465 nm and 665 nm. The quotient of absorbance at 465 nm and that at 665 nm is defined as E_4/E_6.

Infrared spectra

Copper complexes with WSOF were prepared by shaking the WSOF in 0.1M $Cu(ClO_4)_2$ (10:1 wt/wt). The WSOF samples were dried for 1 hour at 323 K and 20 min at 378 K prior to preparation of KBr pellets. Approximately 0.5 mg of WSOF (dried) and 250 mg of KBr were mixed with a mortar and pestle and transferred to a KBr disc press, which was evacuated for 2 min. Infrared spectra were recorded from 4000 to 200 cm^{-1} with a blank KBr pellet in the reference beam. All samples were stored in a desiccator over P_2O_5 before infrared analysis. Infrared

spectra were recorded with a Perkin-Elmer model 457 double beam grating infrared spectrophotometer.

^{1}H-NMR spectra

The concentrated WSOF (2 mL, 4 g L^{-1}) was dried for 3 hours at 343 K, dissolved in 2 mL of D_2O ('Baker'™ Grade, min. 99.75 atom %D), redried at 343 K to constant weight over P_2O_5 under vacuum, and then redissolved in 1 mL of D_2O. All sample preparation was conducted in a N_2 atmosphere to avoid contamination with atmospheric water. The ^{1}H-NMR spectra were recorded on a Varian FT80A NMR spectrometer using a 5-mm NMR sample tube, with tetramethylsilane (TMS) as an external standard at 296±2 K. The instrument was operated in the Fourier transform mode, with a D_2O lock, spin rate (20 rps), spectral width (1202 Hz), no. of transient (300), acquisition time (2.5S), pulse width (18 μsec.), data points (6010), transmitter offset (46), and receiver gain (4).

RESULTS AND DISCUSSION

Yields (Table 2) of WSOF samples were low compared to those of fulvic acids extracted from sludge-soil mixtures (Holtzclaw et al., 1976). The total metal contents of the purified WSOF samples (Table 1) were also low as compared to the total metal contents of sludge-soil mixtures (Holtzclaw et al., 1976), indicating the purifying effect of the cation exchange resin and pH adjustment to 10.5 .

The SS had the highest percentages of H, N, and P, over twice that found in W, while the relative percentages of H, N, and P for WS_6 and WS_1 were intermediate (Table 2). The P content in fulvic acid extracted from sewage sludge (Sposito et al., 1976) ranged from 0.34

Table 1. Analytical data of water soluble organic fractions.

Elements	W	WS_6	WS_1	SS
	mol/g WSOF†			
Na_T	$<6.5 \times 10^{-6}$	2.4×10^{-4}	3.8×10^{-4}	3.8×10^{-5}
K_T	2.3×10^{-5}	3.8×10^{-5}	8.0×10^{-5}	2.5×10^{-5}
NH_4^+	1.7×10^{-4}	2.0×10^{-4}	5.6×10^{-4}	8.2×10^{-4}
Ca_T	$<3.8 \times 10^{-6}$	$<2.6 \times 10^{-6}$	$<5.0 \times 10^{-6}$	$<4.4 \times 10^{-7}$
Mg_T	$<6.2 \times 10^{-6}$	$<4.2 \times 10^{-6}$	$<8.2 \times 10^{-6}$	$<7.2 \times 10^{-7}$
Ni_T	$<2.6 \times 10^{-6}$	$<1.7 \times 10^{-6}$	3.4×10^{-6}	3.0×10^{-7}
Cu_T	1.2×10^{-4}	1.5×10^{-4}	1.6×10^{-4}	2.2×10^{-5}
Cd_T	$<1.3 \times 10^{-6}$	$<9.1 \times 10^{-7}$	$<1.8 \times 10^{-6}$	1.6×10^{-7}
Mn_T	2.8×10^{-6}	9.3×10^{-6}	1.8×10^{-5}	6.4×10^{-7}
Fe_T	2.4×10^{-5}	4.2×10^{-5}	6.4×10^{-5}	8.7×10^{-6}
Cl^-	$<1.5 \times 10^{-6}$	$<1.0 \times 10^{-6}$	2.8×10^{-4}	4.6×10^{-4}
NO_3^-	8.9×10^{-4}	1.3×10^{-4}	5.7×10^{-4}	4.4×10^{-4}
P_T	2.2×10^{-5}	2.6×10^{-5}	3.2×10^{-5}	1.3×10^{-4}
N_T	1.6×10^{-3}	2.2×10^{-3}	3.2×10^{-3}	3.9×10^{-3}
C_T	3.4×10^{-2}	3.0×10^{-2}	4.0×10^{-2}	3.8×10^{-2}
S_T	5.6×10^{-4}	1.2×10^{-3}	1.3×10^{-3}	7.8×10^{-4}

† Moisture and ash-free basis.

Table 2. Elemental analysis and yield of water soluble organic fractions.*

WSOF	Ash	Yield	C	H	N	S	O	P	H/C	C/N
	%†	----------%‡----------								
W	15.5	0.033	41.2	3.7	2.3	1.8	51.0	0.069	1.1	20.9
WS_6	12.7	0.049	36.5	5.4	3.1	3.8	51.2	0.082	1.8	13.7
WS_1	7.3	0.025	48.3	6.8	4.5	4.1	36.3	0.100	1.7	12.5
SS	10.7	0.057	45.2	7.5	5.4	2.5	39.4	0.410	2.0	9.8

†Moisture-free basis.

‡Moisture and ash-free basis.

*Determined in triplicate.

Table 3. Functional group content and E_4/E_6 of water soluble organic fractions.

WSOF	Total acidity	COOH	Phenolic OH‡	E_4/E_6
	----------mol/kg WSOF†----------			
W	3.2	2.1	1.1	10.7
WS_6	4.7	3.5	1.2	10.0
WS_1	9.7	5.1	4.6	4.3
SS	10.9	4.8	6.1	2.8

†Moisture and ash-free basis.

‡Phenolic OH = total acidity - COOH.

to 2.13%, with the lower value being comparable with that observed in the SS.

The major differences in the composition between sewage sludge fulvic acid and soil organic matter fulvic acid may be the high percentage of S in the former, which ranged from 4.5 to 11.6% (Sposito et al., 1976). The S content of the SS was much lower than the S reported in the fulvic acid (Table 2). Sulfone groups may be the predominant form of organic S in the sewage sludge fulvic acids (Sposito et al., 1976). In the present study abundant foaming was observed and unique smells occurred after the liquid fraction of sewage sludge was treated with H^+-saturated resin during the purification procedure. These properties may be related to the presence of unaltered anionic surfactants; however, the S content of SS was only 1.5 times higher than that of the W, but lower than that of WS_6 or WS_1 (Table 2). The major S functional group in SS may be the sulfonyl group in SO_3H (Holtzclaw and Sposito, 1978), while SO_4^{2-} may be the major form in W.

The WS_6 was closer to W in its analytical properties and WS_1 more closely related to SS. Sewage sludge added to the soil should be degraded and probably humified during the aging time (1-6 years), while sludge incubated with soil for 1 week may still have its basic original character, thus explaining the similarity in analytical properties between WS_6 and W.

The C/N ratio of the WSOF decreased as sludge was added to the soil because the sludge application increased the N concentration (Table 2). The fulvic acid extracted from sewage sludge (Sposito et

al., 1978) contained a N content of 3.1% and a C/N ratio of 17.0, higher than SS.

The sludge application increased the H concentration and thus the H/C ratio (Table 2). The H/C ratio of the fulvic acid extracted from Rialto sewage sludge (Sposito et al., 1976) was 1.9, closely resembling the SS. From the H/C data (Table 2), the SS may have a more saturated aliphatic character while W may have a relatively unsaturated, probably, aromatic character.

Generally, the sludge application increased the total acidity, COOH, and phenolic OH in the WSOF (Table 3). Functional group contents (total acidity, COOH, phenolic OH) of humic acid (HA) extracted from soil were higher than those from sewage sludge (Boyd et al., 1980). Therefore, water soluble organic materials may be quite different from HA extracted with alkali and mineral acid. The "model" soil fulvic acid (Schnitzer, 1978) gives a value of 10.3 (mol/kg) for total acidity, 8.2 for COOH, and 3.0 for phenolic OH, higher than W. The carboxyl group content of fulvic acid extracted from the sludge-soil mixture ranged from 0.6 to 4.6 mol/kg (Holtzclaw and Sposito, 1979), which is comparable with the 4.8 mol/kg of SS (Table 3). Therefore, the functional groups are different depending on the source of the organic matter and preparation methods employed.

Based on elemental composition and functional group content, SS appeared to be more aliphatic, N-rich with more carboxyl groups and phenolic hydroxyls as compared to W.

The UV-visible spectra of W, WS_6, and WS_1 samples were generally featureless (Fig. 2). However, the SS showed a weak absorption band at about 260 nm. This absorption has been reported in fulvic acid

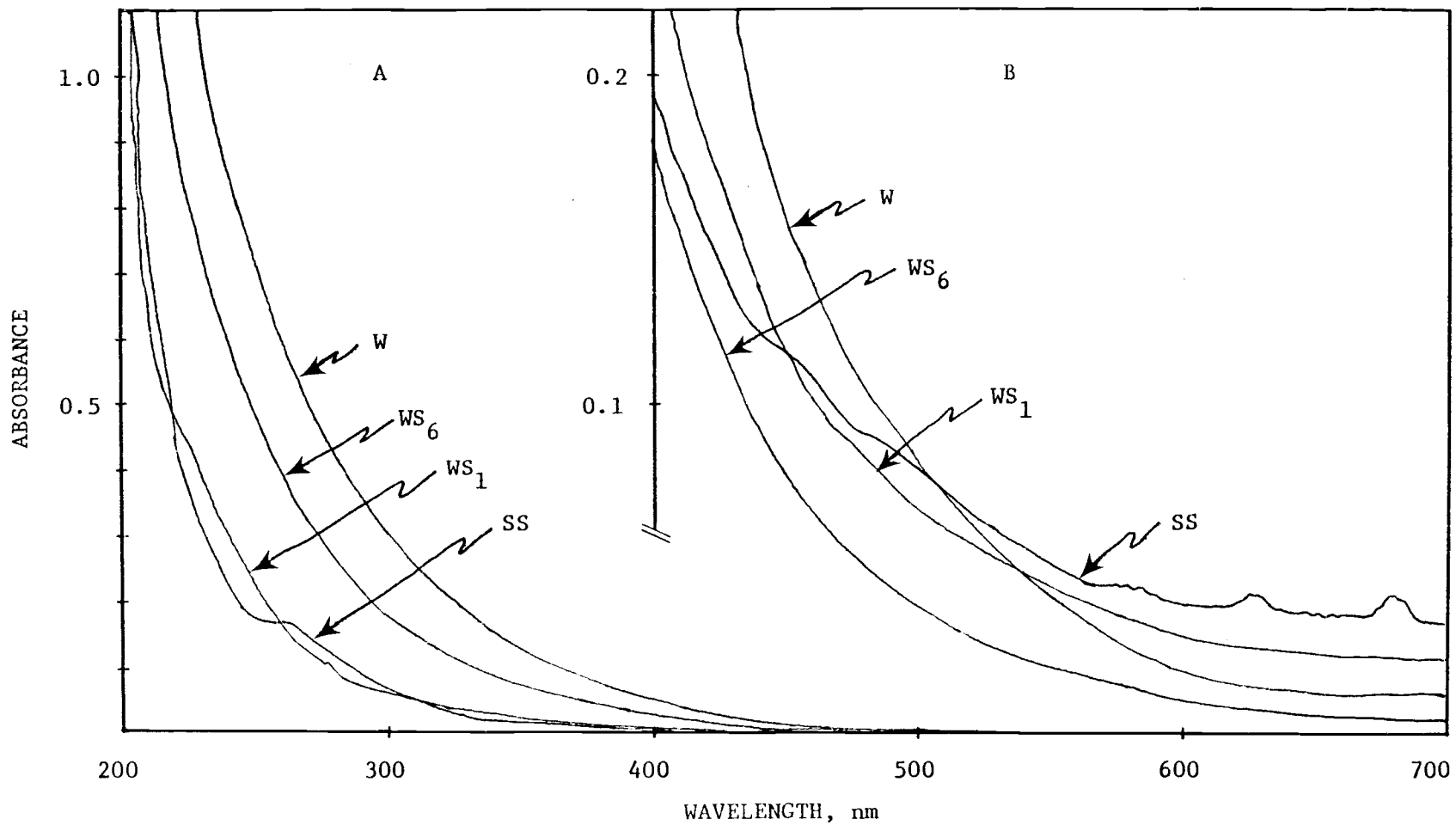

Figure 2. The UV-visible spectra of water soluble organic fractions. [WSOF] g L^{-1}: A,0.05 ; B,0.50

extracted from sludge-soil mixture (Sposito et al., 1976) and assigned to an aromatic character since a peak at 265 nm had been observed in highly aromatic fractions isolated from soil organic matter fulvic acid and humic acid (Schnitzer and Skinner, 1968a; Khan and Schnitzer, 1971). Although the spectra of W and WS_6 are featureless (Fig. 2), their absorptivities at 260 nm are higher than that of WS_1 or SS; therefore, W may have the highest aromatic character, WS_6 intermediate, and WS_1 or SS the lowest. These data are correlated with the H/C ratios which suggest the same trend in degree of unsaturation.

Infrared spectra of all WSOF samples were dominated by strong, broad multi-absorption bands near 1100 cm^{-1} (Fig. 3). The IR spectra of W and WS_6 were generally similar to those in the Type II spectra, and the spectra of WS_1 and SS resembled those in the Type III spectra of soil organic matter fulvic acids (Stevenson and Goh, 1971). Type II spectra were distinguished by a strong band at 1720 cm^{-1}, a second one centered near 1640 cm^{-1}, and the absence of a 1600 cm^{-1} band. Type III spectra were characterized by strong absorption bands near 1540 and 1050 cm^{-1} in addition to major bands shown by Type II. The IR spectra of fulvic acid from the sludge-soil mixture (Sposito et al., 1976) were generally similar to a Type III spectrum.

The 3440 cm^{-1} band in all WSOF samples was rather strong and broad and probably arises from H-bonded OH stretching (Sposito et al., 1976; Tan et al., 1971; Chen et al., 1978). In some cases, N-H stretching may also contribute to absorption in this region (Sposito et al., 1976). Absorption in the 2920 cm^{-1} region (aliphatic C-H stretching) is relatively weak, indicating the absence of large amounts of CH_2 and CH_3 groups (Sposito et al., 1976; Baham et al.,

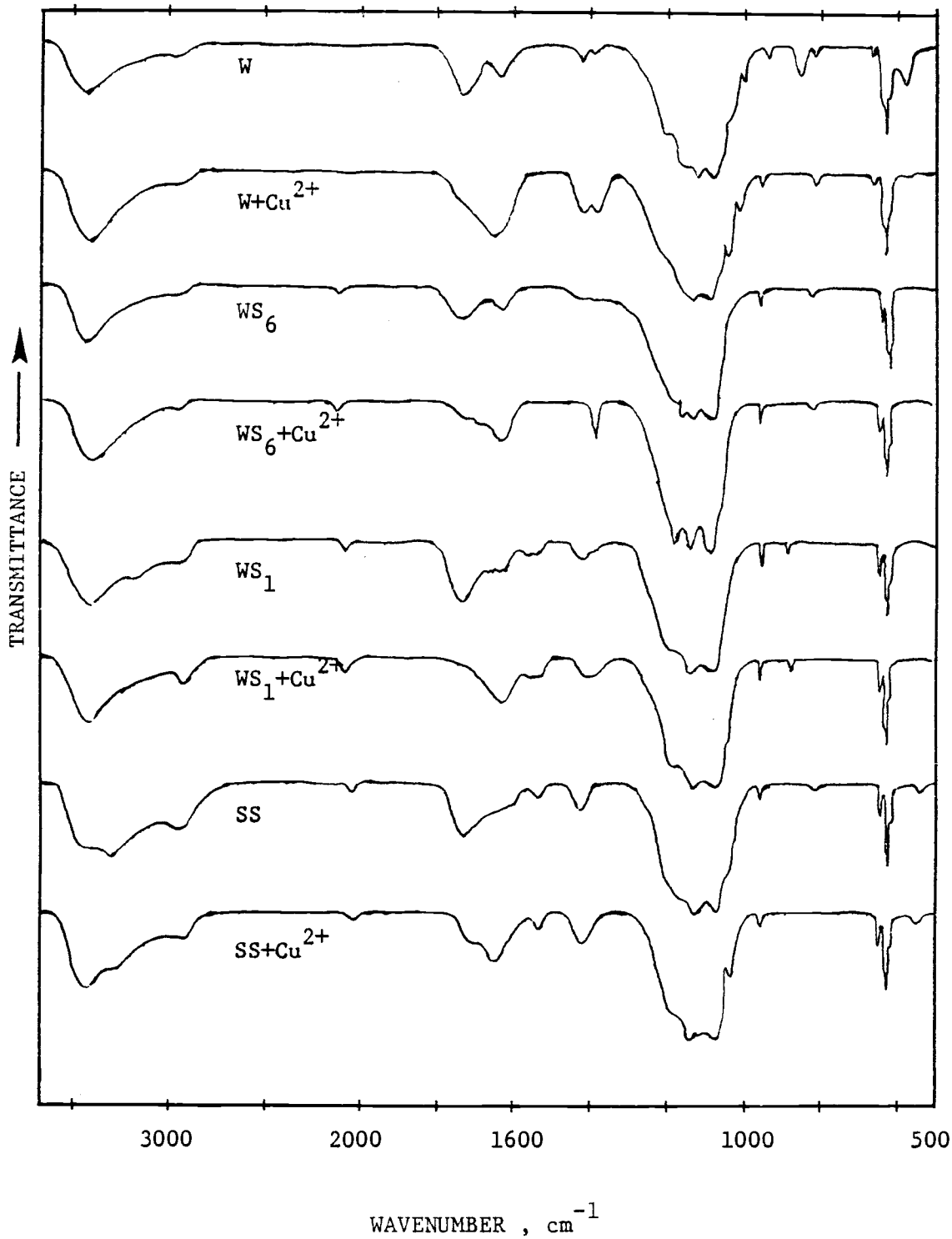

Figure 3. Infrared spectra of water soluble organic fractions and Cu(II)-WSOF complexes.

Table 4. Infrared band assignments for water soluble organic fractions.

W	Absorption, cm^{-1} WS_6	WS_1	SS	Assignment	Ref.
3440(S)†	3440(S)	3440(S)	3440(Sh)	Hydrogen bonded OH stretching, NH stretching	10, 35, 39
--	--	--	3300(S)	Polymeric OH (bonded), NH stretching	3
--	--	3160(Sh)	--	NH stretching of secondary amide (bonded NH)	3
2920(W)	2920(W)	2920(W)	2920(M)	Aliphatic CH stretching	2, 10, 35
--	2065(W)	2060(W)	2030(W)	Amino-acid hydrochloride	3
1720(S)	1720(M)	1720(S)	1720(S)	C=O stretching of COOH, C=O stretching of ketonic carbonyl	3, 10
1630(M)	1630(M)	1630(M)	1650(M)	COO^-	3, 10
--	--	1560(W)	1540(W)	NH deformation (amide II)	35, 37
1420(W)	1420(Sh)	1420(M)	1420(M)	OH deformation and C-O stretching of phenolic OH	37
1390(W)	1385(Sh)	1400(W)	--	COO^-	3, 10
1210(Sh)	1200(Sh)	1200(Sh)	1200(Sh)	C-O stretching and OH deformation in COOH	37
1140(Sh)	1145(Sh)	1145(Sh)	1140(Sh)	Si-O of silicate (ν_3), Si-O-C,	2, 3, 7,
1110(VS)	1115(VS)	1110(VS)	1115(VS)	SiO_2, $SO_4^{2-}(\nu_3)$, $PO_4^{3-}(\nu_3)$,	10, 23,
1190(VS)	1090(VS)	1090(VS)	1090(VS)	S=O symmetric in sulfone group	27, 35
1040(Sh)	[1040]‡	1040(Sh)	1040(Sh)	C-O stretching of polysaccharide	8, 35
1010(W)	[1010]	[1010]	[1010]	P-O-C (aliphatic organic phosphate), phosphate (ν_1)	3, 23
942(W)	942(W)	942(W)	942(W)	ClO_4^- (ν_1)	23
865(M)	--	878(W)	--	Hydrogen bonded OH deformation in COOH	3
830(VW)	838(VW)	--	--	$SiO_4^{2-}(\nu_1)$	23
670(VW)	655(M)	655(M)	655(M)	O-S-O	10
638(Sh)	638(Sh)	638(Sh)	638(Sh)	$SiO_4^{2-}(\nu_4)$, $SO_4^{2-}(\nu_4)$,	
630(S)	630(S)	630(S)	630(S)	$ClO_4^-(\nu_4)$	23
620(Sh)	615(Sh)	615(Sh)	615(Sh)		
580(M)	--	--	--	COOH rocking	4, 35
--	--	--	535(W)	$PO_4^{3-}(\nu_4)$, $SiO_4^{2-}(\nu_2)$	23

†VS, very strong; S, strong; M, medium; W, weak; VW, very weak; Sh, Shoulder.
‡[ ] unresolved.

1978; Chen et al., 1978). However, the relative intensity of the 2920 cm^{-1} band is highest for SS and lowest for W, supporting the idea that SS has a more aliphatic character.

The 1720 cm^{-1} band was greatly reduced in intensity when the WSOF samples were converted to the copper(II)-carboxylate complexed form by adding $Cu(ClO_4)_2$, and bands near 1630 and 1390 cm^{-1}, which are characteristic of the carboxylate ion (COO^-), were increased. These results agree with other studies indicating that the 1720 cm^{-1} band is due largely to the C=O of COOH (Bellamy, 1975; Chen et al., 1978). The shoulder at 1720 cm^{-1} in the spectrum of the copper(II)-carboxylate complexed forms indicates the presence of a small amount of ketonic or aldehydic C=O. In the case of WS_1 and SS giving Type III spectra, the band near 1550 cm^{-1} is attributed to the NH deformation (amide II) of the peptide linkage of proteins (Sposito et al., 1976; Stevenson and Goh, 1971).

Definite assignments cannot be given for bands in the 1090-1145 and 615-638 cm^{-1} regions. The strong multiple bands near 1100 cm^{-1} in the spectra of humic and fulvic acids have been attributed to one or more of the following: S=O in sulfone group (Sposito et al., 1976); S=O symmetric (Chen et al., 1978); SO_4^{2-}, PO_4^{3-}, SiO_4^{2-}, and ClO_4^-, (Nakamoto, 1963); sulfonate ion (Baham et al., 1978); quartz, SiO_2 (Boyd et al., 1979); Si-O of silicate (Schnitzer, 1978); Si-O-C (Bellamy, 1975). The shoulder and sharp bands near 630 cm^{-1} are probably due to $SiO_4(\nu_4)$, $SO_4^{2-}(\nu_4)$, and $ClO_4^-(\nu_4)$ (Nakamoto, 1963). The presence of ClO_4^- was due to the addition of $HClO_4$ to convert the WSOF into the H-form or to adjust pH values during the sample preparation.

The absorption band near 1040 cm^{-1} which is characteristic of C-O stretching of polysaccharides (Sposito et al., 1976; Boyd et al., 1980) was not resolved clearly due to the numerous overlapping absorptions in this region. The shoulder absorption band may still indicate the presence of polysaccharides in WSOF samples.

The band near 580 cm^{-1} is interpreted as a rocking mode of a carboxyl group attached to a benzene ring (Bentley et al., 1968, p. 69). If this mode in fact contributes to the absorption at 580 cm^{-1}, it attests to the aromatic character of W.

The absorption band near 870 cm^{-1} may be assigned to the carboxyl group, in particular, to the deformation of an H-bonded OH (Bellamy, 1975). Since the band does arise from a deformation mode, it is expected to undergo a change if the H bond in which the OH group participates is disrupted. In the absence of H bonding, the band disappears (Bellamy, 1975, p. 197). Indeed, when Cu^{2+} was added, the band disappeared in W and WS_1 (Fig. 3) suggesting that the OH group in COOH may be ionized when the carboxyl binds a metal ion.

The band near 2050 cm^{-1} which was assigned to amino acid hydrochlorides (Bellamy, 1975) appeared only in sludge or sludge-derived water soluble organic fractions (Fig. 3). The presence of protein or protein decomposition products has been confirmed in the sludge or sludge-soil mixtures (Sposito et al., 1976).

The most important common features of water soluble organic fractions from soil and sludge or sludge amended soils are: (i) a fairly weak absorption at 2920 cm^{-1} due to aliphatic CH_2 and CH_3, (ii) relatively similar absorption bands at 1720 cm^{-1} (C=O stretching of COOH or C=O stretching of ketonic carbonyl), and 1650 cm^{-1} (COO^-),

(iii) shoulder absorption bands at 1210 cm^{-1} (C-O stretching and OH deformation in COOH) and 1040 cm^{-1} (C-O stretching of polysaccharides), and (iv) strong broad multi-absorption bands at 1090-1145 cm^{-1} (Si-O of silicate, SO_4^{2-}, PO_4^{3-}, S=O in sulfone group, SiO_2, ClO_4^-), and strong bands near 630 cm^{-1} (SiO_4^{2-}, SO_4^{2-}, ClO_4^-).

The most important differences among the WSOF samples are: (i) SS and WS_1 have broad absorption bands near 3160-3300 cm^{-1} (bonded N-H stretching of secondary amide), and a rather pronounced absorption band near 1550 cm^{-1} (NH deformation of secondary, non-cyclic amides in peptide linkage of protein), (ii) SS, WS_1, and WS_6 have an absorption band near 2050 cm^{-1} (amino acid hydrochloride), (iii) W has an absorption band at 580 cm^{-1} (a rocking mode of a carboxyl group attached to a benzene ring), and (iv) SS and WS_1 have higher absorption intensities than W at 1420 cm^{-1} (OH deformation and C-O stretching of phenolic OH).

The principal conclusions to be drawn from the IR data are: (i) W has more aromatic carboxyl groups, (ii) SS has more aliphatic carboxyl and phenolic hydroxyl groups, and (iii) SS or WS are characterized by the presence of protein decomposition products.

Nuclear magnetic resonance (^{1}H-NMR) bands centered near 0.75, 1.10, and 1.7 ppm (Fig. 4) indicate protons on methyl terminal groups of aliphatic chains, while the resonances near 2.30 ppm indicate protons on carbons adjacent to carbonyl groups (Dyer, 1965). All WSOF samples had resonance bands near 0.75 and 1.7 ppm; however, a higher absorption intensity at 1.7 ppm was observed in SS, suggesting that SS had more aliphatic protons than the other WSOF samples. The bands near 1.30 ppm indicate protons on the secondary alkyl amines (Dyer,

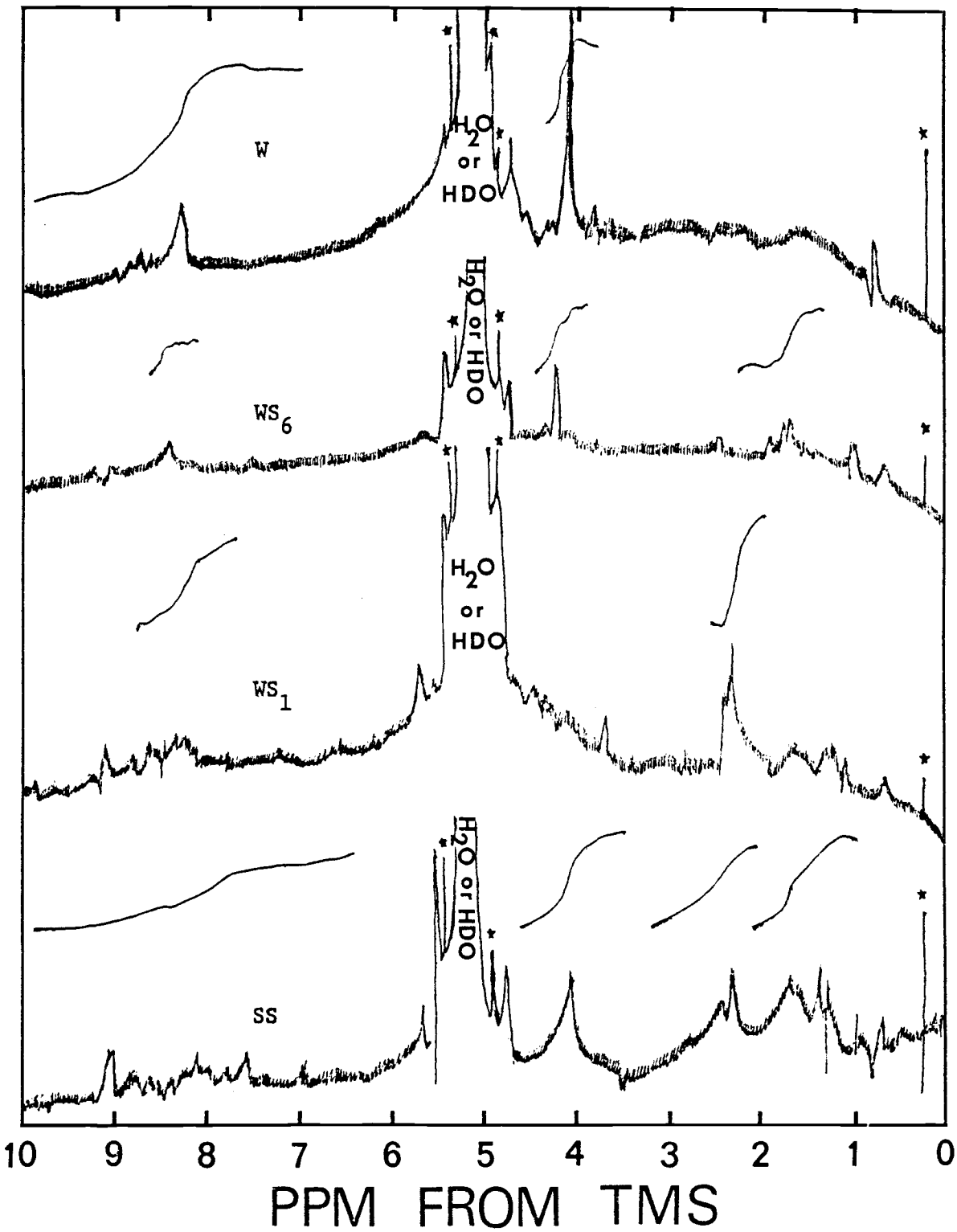

Figure 4. ^{1}H-NMR spectra of water soluble organic fractions dissolved in D_2O. Features marked with asterisk are spinning sidebands due to intense H_2O-HDO and TMS resonance.

Table 5. ^{1}H-NMR band assignments for water soluble organic fractions.

Chemical shift (δ), ppm					
W	WS_6	WS_1	SS	Structural type	Ref.
0.80(W)†	0.75(W)	0.75(W)	0.75(W)	CH_3-C- (saturated)	11
--	1.05(W)	1.10(W)	--	CH_3-C-C-X*	11
--	--	1.25(W)	1.30(M)	R_2NH	11
1.7 B(W)	1.65B(W)	1.65B(W)	1.7 B(M)	CH_3-C-X**	11
--	--	2.40(M)	2.30(M)	CH_3-C=O	11
--	--	--	2.45B(W)	CH_3-S-, CH_3-N , CH_3Ar	11
--	--	3.75(W)	--	ArSH, CH_3-O-, $ArNH_2$, ArNHR	11
4.15(S)	4.25(M)	4.15(W)	4.10(M)	ROH	11, 17, 25, 26
4.75(W)	4.75(W)	--	4.80(M)	CH_2=C (nonconjugated)	11
5.52(W)	5.42(M)	5.50(M)	5.52(M)	ArOH	5, 11
--	5.75(W)	5.75(W)	5.65(W)	CH_2=C (conjugated)	11
8.35B(W)	8.45B(W)	8.25-8.75B(W)	7.6 B(W), 8.1 B(W)	ArH, H-N-C(=O)-	11, 36
--	--	9.15(W)	8.8 B(W), 9.03(W)	$ArNH_3^+$, $ArRNH_2^+$, ArR_2NH^+	11

†W, weak; M, medium; S, strong; B, broad.

* X = Cl, OH, OR, C=O, N

** X = Cl, OH, OR, OAr, N

1965). The SS and WS_1 had a resonance band in this region; however, SS showed higher absorption suggesting that SS had a significant amount of protein decomposition product. The resonances near 4.15 ppm for all WSOF samples may be assigned primarily to protons in polysaccharide decomposition products (James, 1975; Dyer, 1965; Sadtler Res. Labs, 1969, 1976).

The resonances near 5.50 ppm may be related to phenolic hydroxyl protons in polymeric association (Dyer, 1965; Bible, 1967). The SS showed relatively stronger absorption intensity in this region indicating that SS has the highest phenolic hydroxyl group content. This result is supported by the functional group analysis and IR spectra.

The broad resonances in the range 7.6-8.7 ppm which are characteristic of an aromatic proton (Sposito et al., 1978; Dyer, 1965) indicate that sludge-derived WSOF samples which show broader complicated bands have a more complex variety of aromatic protons than the soil WSOF which shows a relatively narrower band in this region. The SS-protons have a wider variety of structural environments than W-protons (Fig. 4 and Table 5).

Principal conclusions from the ^{1}H-NMR spectra of WSOF samples are: (i) SS has more aliphatic protons than W, (ii) SS has pronounced amounts of secondary alkyl amine protons, not in common with W, (iii) sludge-derived WSOF samples have more phenolic hydroxyl protons than W, (iv) structural complexities around the aromatic protons followed the following order: SS > WS_1 > WS_6 > W, and (v) SS-protons have a wider variety of structural environments than W-protons.

CONCLUSIONS

The SS was characterized by higher contents of H, N, and P, higher H/C ratio, and lower C/N ratio than that of W. Total acidity, carboxyl and phenolic hydroxyl group contents were also higher in SS than in W. On the other hand, the overall aromatic character and aromatic carboxyl group contents were higher in W than the other WSOF samples. Aliphatic carboxyl and proton contents were higher in SS than in W. Structural complexities around the aromatic protons followed the following order: SS > WS_1 > WS_6 > W. The SS-protons had a wider variety of structural environments than W-protons. Protein decomposition products were more evident in SS than in W.

The elemental and functional group contents of WS_6 and WS_1 were generally intermediate between W and SS, with WS_6 closer to W and WS_1 more closely allied to SS. Therefore, sewage sludge amended to the soil appears to degrade and humify during the aging time (1-6 years).

The SS has generally similar contents of C and H, higher N and COOH contents, and a lower S content than fulvic acid extracted from sewage sludge. The W has a lower C and H content, similar N and S contents, and a much lower total acidity and COOH content than the "model" soil fulvic acid. Therefore, SS appears to contain more proteinaceous material with a higher carboxyl group content than fulvic acid extracted from sewage sludge, and W may have less O-containing functional groups, suggesting that WSOF may be quite different from fulvic acid extracted with an alkali solution.

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Copper(II) Binding Mechanisms with Water Soluble Organic Fractions Extracted from Sewage Sludge Amended Soils[1]

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ABSTRACT

Cu(II) binding mechanisms with water soluble organic fractions (WSOF) extracted from an agricultural soil (W), a soil treated with sludge for 6 years (WS_6), a sludge-soil mixture incubated for one week (WS_1), and sewage sludge (SS) were studied by electron spin resonance (ESR) spectroscopy and potentiometric titrations.

Cu(II)-WSOF complexes produced $g_{\parallel}$ values which were larger than $g_{\perp}$ values, indicating that the coordination of Cu(II) complex was an elongated octahedron. At liquid N_2 temperature (77K), the Cu(II)-W complex showed an anisotropic ESR spectrum while the Cu(II)-SS complex showed an isotropic spectrum. These spectral results suggest that the oxygen donor ligands of W may form relatively strong bonds with Cu^{2+} due to extensive chelation while ligands of SS may form little or no chelate bonds with Cu^{2+}. The ESR spectra of Cu(II)-SS complex also

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suggest that each of four in-plane ligands (e.g., COO^-, H_2O, Cl^-, etc.) may act independently as monodentate ligands.

Oxygen donor ligands such as aromatic carboxyl groups were probably the major Cu(II) binding sites in W. Sulfonate, aliphatic carboxyl group, and N-containing ligands were probably the major binding sites in SS at pH 5. The Cu(II) complexation with N-containing groups increased as sludge was added to the soil.

Much higher (6x) pyridine concentrations were required to displace W from Cu(II)-W complex as compared to the Cu(II)-SS complex.

Additional Index Words: electron spin resonance, chelation, binding sites, ligand exchange, humic materials.

INTRODUCTION

Complexation of Cu^{2+} with humic acid (HA) and fulvic acid (FA) extracted from soils has generally been considered to involve chelation by o-hydroxycarboxylic acid and o-dicarboxylic acid moieties, the former being predominant (Schnitzer and Khan, 1972; Stevenson and Ardakani, 1972; Van Dijk, 1971). Metal ions, however, may complex with the same oxygen-containing functional groups without forming a chelate ring.

The stereochemistry and mechanisms of Cu^{2+} binding to soil HA and peat has been studied (McBride, 1978; Bloom and McBride, 1979). They concluded from ESR spectral data that the negatively charged surface functional groups of soil HA and peat might enter axial (z-axis) positions, rather than displace strongly bound H_2O ligands in the xy plane of the Cu^{2+} ions due to the Jahn-Teller distortion. McBride (1978)

also observed no direct evidence for the formation of more than a single bond between the HA and Cu^{2+}, although chelation could not be eliminated as a mechanism in the complexation of Cu^{2+}. Bloom and McBride (1979) concluded that chelation of Cu^{2+} with 2-hydroxycarboxylic and 1,2-dihydroxy sites was not significant in soil organic matter. However, this proposed mechanism (axial position binding) might violate the Jahn-Teller theorem in which Cu^{2+} prefers square planar complexation.

A more recent ESR study (Boyd et al., 1981) of Cu(II) binding to soil HA suggested that Cu(II) formed two equatorial bonds with oxygen donor atoms of HA. The addition of pyridine to the Cu(II)-HA complex resulted in the selective displacement of equatorially coordinated H_2O ligands by the N donor ligands without displacement of HA oxygen donor ligands. However, this suggestion is questionable because a direct conclusion cannot be drawn regarding the arrangement of the ligands from the g factor alone and their orientation dependence (Vänngård, 1972, p. 416). Furthermore, the HA employed in their study was of relatively higher molecular weight material (residue on Whatman no. 1 filter paper), which would result in anisotropic ESR spectra at room temperature (Vänngard, 1972).

The objective of this study is to further develop a description of the Cu^{2+} binding with water soluble organic materials in sewage sludge amended soil.

MATERIALS AND METHODS

Water soluble organic fractions (WSOF) were prepared from (i) Willamette silt loam soil (fine-silty, mixed, mesic, Pachic Ultic

Argixeroll), (ii) a soil treated with sludge for 6 years (annually 25 kg/m^2 of sewage sludge and 45 g N/m^2 as $(NH_4)_2SO_4$), (iii) sewage sludge-soil mixture incubated for one week in the laboratory (equivalent to an application rate of 50 kg/m^2 of sewage sludge), and (iv) sewage sludge from Portland, OR (Lim et al., 1982). All discussion of the Cu(II) binding mechanisms will refer to only the WSOF of each sample.

The WSOF (3 mL of 0.033 kg L^{-1}) from each scurce was titrated in a background supporting electrolyte of 0.1M $NaClO_4$ with standardized 0.1M NaOH at 296K after first acidifying the solution to pH 3.00 with $HClO_4$. The titration was performed on a London automatic titrimeter fitted with a Radiometer G2040B glass electrode and a K4040 calomel electrode. The titration rate was 3.0 μL min^{-1}.

Electron spin resonance (ESR) spectra were recorded with a Varian E-4 EPR spectrometer calibrated with α,α-diphenyl-β-picrylhydrazyl (dPPh; g = 2.0037±0.0002). Room temperature spectra were recorded with samples placed in Wilmad quartz flat face aqueous cells. Liquid N_2 temperature (77K) spectra were recorded in Wilmad quartz tubes with samples placed in glass capillary tubes. Ethylene glycol:water (2:1) solvent was used to obtain an anisotropic spectrum of $[Cu(aq)]^{2+}$ at 77K (Walker et al., 1972).

Purified WSOF (Lim et al., 1982) were concentrated to 1 to 4 g L^{-1} on the ash-free basis. Total indigenous soluble Cu contents (Cu_{TS}) of W and WS_6 (Table 7) were sufficient to obtain resolved spectra. Indigenous Cu contents (Cu_{TS}) of WS_1 and SS were not sufficient to produce spectra and additional Cu^{2+} as $Cu(ClO_4)_2$ was added (Fig. 5, Table 7). The ionic strengths of concentrated WSOF samples

Table 6. ESR spectrometer settings.

	Temperature	
	296K	77K
Field set	3050 G	2500 G
Microwave		
frequency	9.521 GHz	9.078 GHz
power	10 mW	10 mW
Scan		
range	2000 G	4000 G
time	8 min	8 min
Modulation		
frequency	100 KHz	100 KHz
amplitude	4 x 10°G	4 x 10°G
Time constant	1.0 S	1.0 S
Receiver gain	4×10^5	10×10^4

were computed from elemental analyses data (Lim et al., 1982) and adjusted to equivalent levels by adding $NaClO_4$. The pH of the samples was adjusted with dilute $HClO_4$ or NaOH. Stepwise additions of 0.1M $H_2C_2O_4$ (ca. pH 1.65), 0.1M $Na_2C_2O_4$, and pyridine were added to the Cu(II)-WSOF complexes to investigate the displacement effect and possible steric conformations of the Cu(II)-WSOF complexes. Sample solutions were shaken at least 5 hours before ESR spectra were measured.

RESULTS AND DISCUSSION

The Cu(II)-W complex showed relatively well resolved anisotropic ESR spectra while the Cu(II)-SS complex showed unorganized isotropic ESR spectra even with a high Cu^{2+} content at liquid N_2 temperature (Fig. 5). The spectral shapes of the Cu(II)-WS_6 and Cu(II)-WS_1 complexes were rather similar to those of Cu(II)-W and Cu(II)-SS complexes, respectively.

The $g_{\parallel}$ values of Cu(II)-WSOF complexes were larger than the $g_{\perp}$ values (Table 7) indicating that the coordination of the Cu(II) complex was an elongated octahedron. If the symmetry was tetragonal but the axial ligands were closer than the four in-plane ligands, the unpaired electron would enter into d_{z^2} orbital and the orbital contribution to the g factor would be small when the magnetic field was along the z axis; consequently, $g_{\parallel}$ should be smaller than $g_{\perp}$ (Vänngård, 1972). According to the Jahn-Teller theorem, the six ligands associated with Cu^{2+} cannot remain in perfect octahedral coordination but must be distorted in some way. The coordination with cupric ion is best regarded as virtually a square planar complex

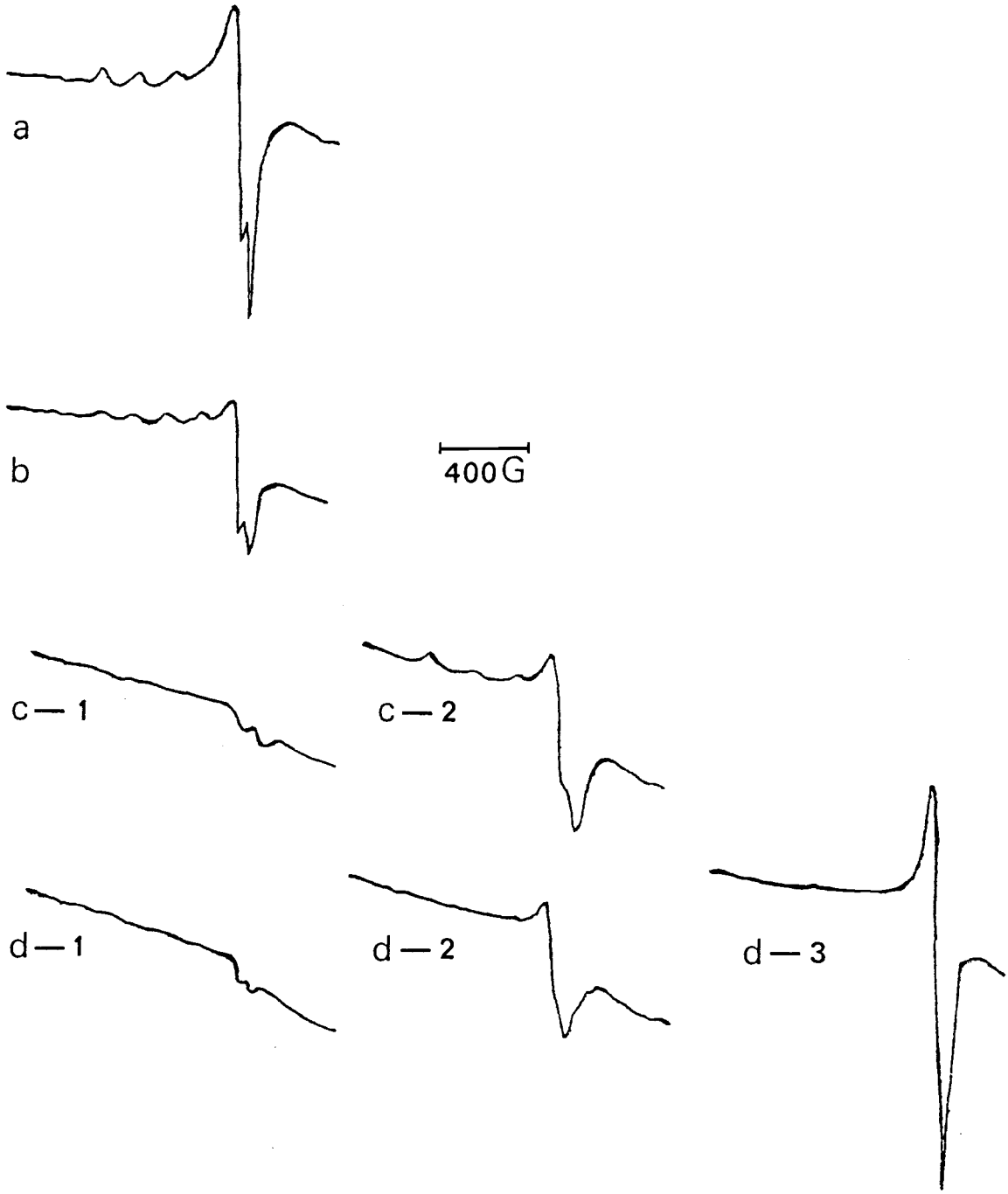

Figure 5. ESR spectra of Cu(II)-WSOF complexes at 77 K. [WSOF]=4×10^{-3}kg L^{-1}, pH=5.50, I=0.01 M. a, Cu(II)-W with $[Cu_{TS}]=7.26\times10^{-4}$mol L^{-1}; b, Cu(II)-WS_6 with $[Cu_{TS}]=6.54\times10^{-4}$ mol L^{-1}; c-1 and c-2, Cu(II)-WS_1 with $[Cu_{TS}]=3.46\times10^{-4}$, and 8.46×10^{-4}mol L^{-1}, respectively; d-1, d-2, and d-3, Cu(II)-SS with $[Cu_{TS}]=3.19\times10^{-4}$, 8.19×10^{-4}, and 5.32×10^{-3}mol L^{-1}, respectively.

Table 7. ESR spectral data for Cu-WSOF complexes at 77K†.

WSOF	Cu_{TS}	$g_{\perp}$	$g_{\parallel}$	$\lvert A_{\parallel} \rvert$
	mol L^{-1}			cm^{-1}
W	7.26×10^{-4}	2.043	2.314	1.84×10^{-2}
WS_6	6.54×10^{-4}	2.041	2.304	1.93×10^{-2}
WS_1	3.46×10^{-4}	2.032	--	--
WS_1	8.46×10^{-4}	2.041	2.258	2.11×10^{-2}
SS	3.19×10^{-4}	2.032	--	--
SS	8.19×10^{-4}	2.046	--	--
SS	5.32×10^{-3}	2.084	--	--

† [WSOF] = 4×10^{-3} kg L^{-1}, pH = 5.50, I = 0.01 M

-- unresolved

(Cotton and Wilkinson, 1972, 1980). Therefore, ligands of WSOF may be coordinated equatorially to the Cu^{2+} center to form a square planar complex. In this case, ligand bindings at the axial positions of the elongated octahedron are not significant.

Orientation dependence (anisotropy) of the Cu(II)-W complex and orientation independence (isotropy) of the Cu(II)-SS complex (Fig. 5) suggest that ligands of W may form relatively strong bonds to Cu^{2+} due to the extensive equatorial chelation while ligands of SS may form little or no equatorial chelate bonds. The ESR spectra of the Cu(II)-SS complex (Fig. 5) also suggest that each of four in-plane ligands (i.e., COO^-, H_2O, Cl^-, etc.) may act independently as monodentate ligands.

The ESR spectral parameters for $[Cu(aq)]^{2+}$ in the (2:1) ethylene glycol:water solvent were $g_{\parallel}$ (2.421), $g_{\perp}$ (2.064), and $|A_{\parallel}|$ (1.58 x 10^{-2} cm^{-1}) at liquid N_2 temperature. The smaller $g_{\parallel}$ values of Cu(II)-WSOF complexes, as compared to $[Cu(aq)]^{2+}$, indicated that crystal-field splitting (Δ) of the oxygen ligand of WSOF was stronger than that of water (Eq. [A.1]). The $g_{\parallel}$ values decreased as sludge was added to the soil (Table 7). A decrease in the $g_{\parallel}$ value implies an increase in the N or S ligand content of Cu(II)-WSOF complexes (Appendix). Indeed, previous analytical data obtained for the W and SS showed the SS contained more N and S-containing functional groups as compared to the suite of organic ligands found in W (Lim et al., 1982).

The $|A_{\parallel}|$ values (Table 7) increased with sludge treatment, indicating that equatorial N or S coordination increased. As shown in Eq. [A.2], the dipole-dipole interaction term (α^2) between the magnetic

moments associated with the spin motion of the electron and the nucleus increases as delocalization of the unpaired electron of Cu^{2+} to the neighboring ligand atom decreases. This is consistent with electronegativities of ligand atoms (O 3.50, N 3.07, S 2.44, obtained from Cotton and Wilkinson, 1972).

The first derivative ESR spectra of the Cu(II)-SS complex showed several absorption peaks which changed their spectral heights depending on the pH of the solution at 296K (Fig. 6). The ESR spectra of the Cu(II)-W complex showed a single absorption (spectra not shown). From the spectra of the Cu(II)-SS complex it appears that more than three types of Cu^{2+}-binding sites may occur in the SS. This apparent heterogeneity of the Cu(II) binding sites is related to the polyfunctional nature of the SS. Conclusions from the ESR spectra are correlated with the potentiometric titration curves which suggest the polyacidic nature of the SS (Fig. 7). Four separate classes of dissociable protons that range in acidity from strong (ionized at pH<2) to weak (ionized at pH>10) have been reported in FA extracted from sewage sludge (Sposito and Holtzclaw, 1977). The strongly acidic groups (probably SO_3H, pK<1) of FA were titrated by KOH below pH 4.5. The other weakly acidic functional groups were: (i) carboxyls titrated in the acidic pH range; (ii) very weakly acidic carboxyls and N-containing groups titrated around pH 7; (iii) phenolic OH and SH groups titrated in the alkaline pH range. Therefore, possible Cu^{2+}-binding sites in a Cu(II)-SS complex are probably dissociated sulfonic acid (sulfonate), carboxylic acid (carboxylate), and N-containing groups in the acid pH range (pH<7).

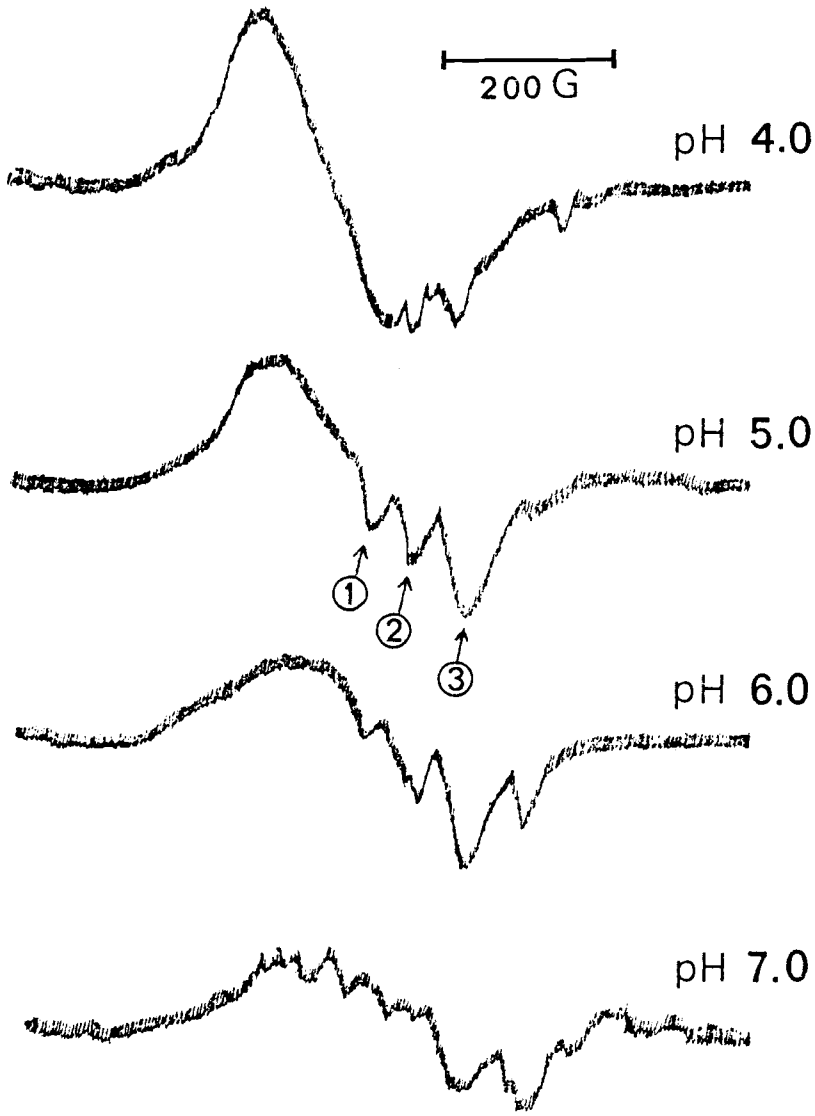

Figure 6. ESR spectra of Cu(II)-SS complexes at 296 K. $[SS]=1.0\times10^{-3}$ kg L^{-1}, $[Cu_{TS}]=2.67\times10^{-4}$ mol L^{-1}, I = 0.01 M.

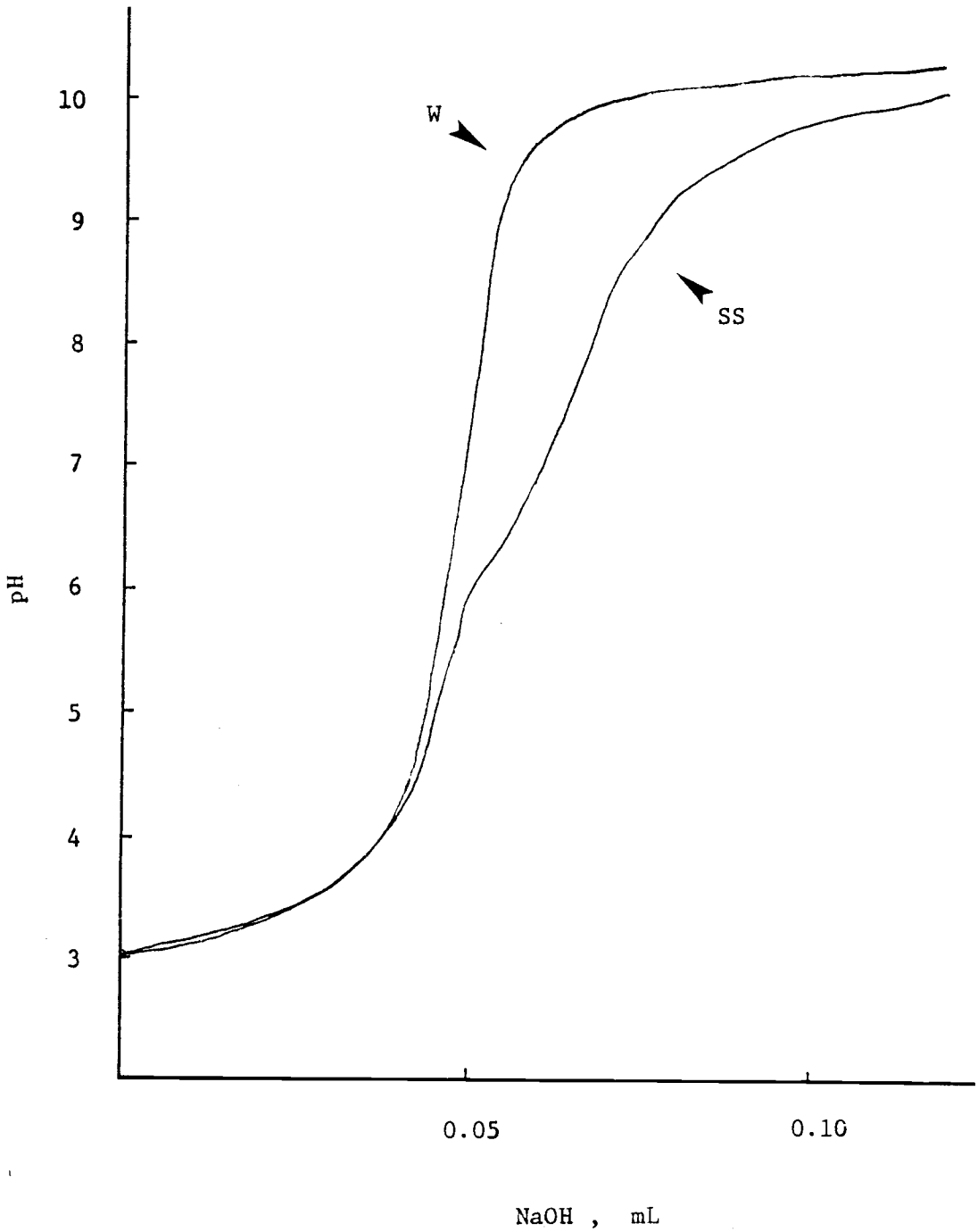

Figure 7. Potentiometric titration curves for W and SS with 0.1 M NaOH. [WSOF]=0.033 kg L^{-1},3 mL ; Initial pH=3.0 ; Ionic background=0.1 M $NaClO_4$; Titration rate=3.0 μL min^{-1} ; Temperature=296 K.

Definite peak identifications in the ESR spectrum of the Cu(II)-SS complex could not be made due to the heterogeneity of the Cu(II) binding sites (Fig. 6). However, if based on the anticipated g values (not calculated, but higher magnetic field yields a smaller g value), the spectrum at pH 5 might be related to free, loosely bound Cu^{2+} ions (arrow 1), oxygen sites such as carboxylate and sulfonate (arrow 2), and N-containing groups (arrow 3).

It is generally supposed that Cu^{2+} ions vibrate between binding sites and solution phase, and exchange of Cu^{2+} ions from binding sites into solution by other Cu^{2+} ions is probably very rapid ($\cong 10^{11} S^{-1}$). However, the time distribution for ions on the binding sites or in the solution phase may vary depending on the bond strength. The height of the ESR spectrum is a linear function of the concentration of free metal ion (Cohn and Townsend, 1954; Gamble et al., 1977). The higher absorption spectrum may indicate a higher concentration of free Cu^{2+} ion, that is, less bonding and, on the other hand, lower absorption may indicate relatively stronger bonding on the site. Therefore, it is suggested that the Cu^{2+} binding on the oxygen sites may be relatively stronger than that on N-containing groups at pH 5 (Fig. 6). These suggestions are supported by Sposito et al. (1979), who studied cupric ion complexation by FA extracted from sewage sludge. A relatively strong 1:1 complex ($\log^{c}K=3.9$) was formed between Cu^{2+} and the most acidic functional groups in FA and a weaker set of complexes ($\log^{c}K=2$) was formed between Cu^{2+} and the more weakly acidic functional groups.

As mentioned earlier, the Cu(II)-W complex showed an anisotropic spectrum at 77 K (Fig. 5). When 0.1$\underline{M}$ $Na_2C_2O_4$ solutions were added to

the Cu(II)-W complex solution, the anisotropic spectra of the Cu(II)-W complex were not changed, but rather better resolved anisotropic spectra were produced (spectra not shown). In this case, the oxalate ion might act as a bidentate ligand and form a relatively strong chelate bond with Cu^{2+}. However, when 0.1M $H_2C_2O_4$ solutions were added, the anisotropic spectra of the Cu(II)-W complex were changed to the isotropic spectra (Table 8, Fig. 8) which were very similar to those of Cu(II)-SS complexes (Fig. 5). Oxalic acid is a relatively weak acid (pK_1=1.23, pK_2=4.19) and produces a bioxalate ion ($HC_2O_4^-$) by dissociation. Therefore, the Cu^{2+} ion might form Cu(II)-bioxalate in the oxalic acid solution. In this case, the bioxalate ion might act as a monodentate ligand.

Another possible explanation for the different bonding mechanisms between Cu(II)-W and Cu(II)-SS complexes is that W has mainly aromatic carboxyl groups as the major possible binding sites while SS has aliphatic carboxyl groups (Lim et al., 1982). Therefore, steric conformation of ligands in W may favor the formation of a chelate ring with a Cu^{2+} center because ligands (e.g., o-hydroxycarboxylic acid, o-dicarboxylic acid) may act as bidentate units. On the other hand, aliphatic carboxyl groups in SS may be relatively distant from one another and, as a consequence, act independently as monodentate ligands.

As mentioned earlier, ESR spectra (Fig. 5) of the Cu(II)-WS_6 and Cu(II)-WS_1 complexes closely resembled those of Cu(II)-W and Cu(II)-SS complexes, respectively, with respect to the spectral shapes and parameters. The Cu(II) binding mechanism with WS_6 appears similar to the

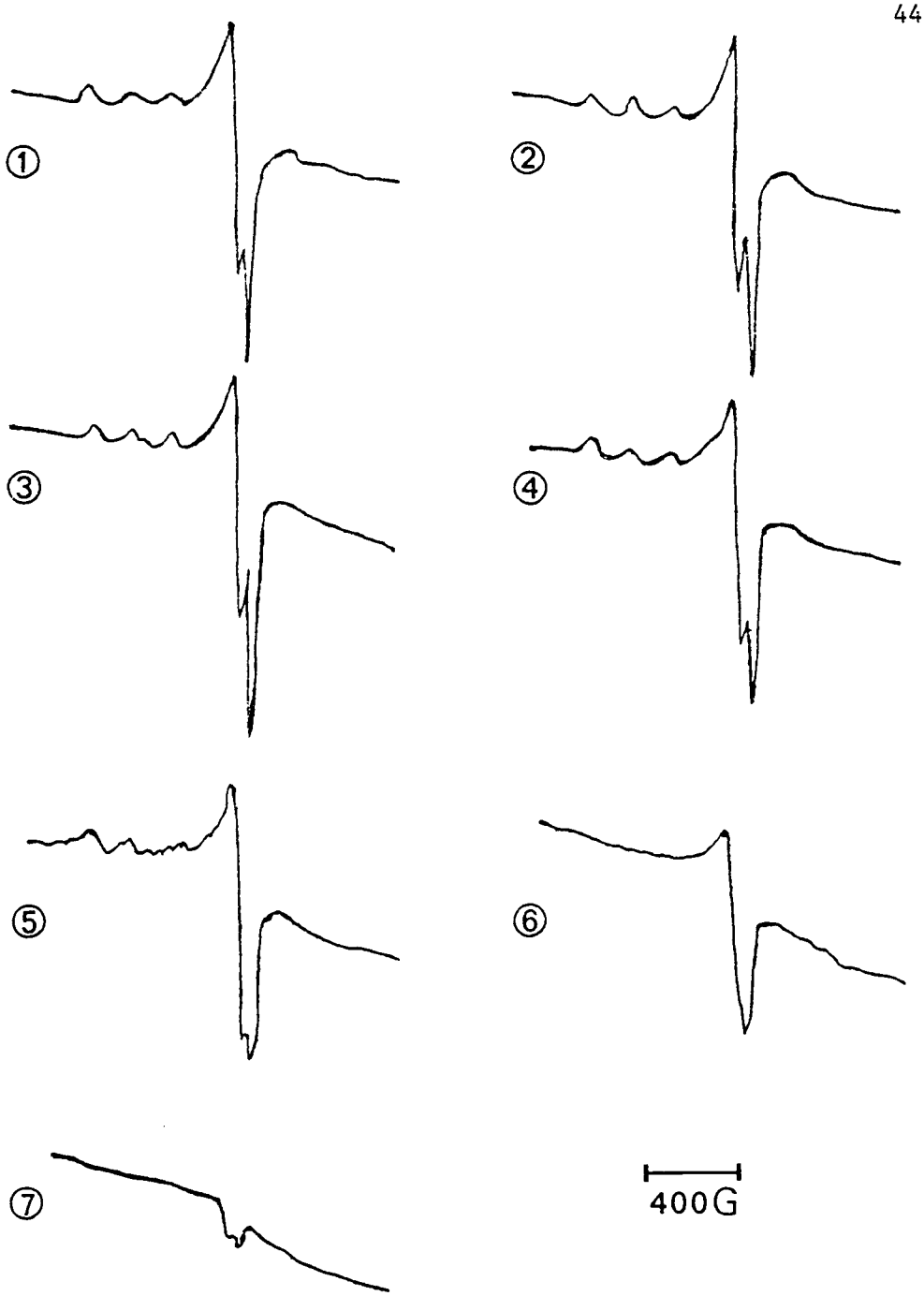

Figure 8. ESR spectra of Cu(II) complexes with W and oxalic acid at 77 K. [W]= $4x10^{-3}$ kg L^{-1}, [Cu_{TS}]=$7.26x10^{-4}$ mol L^{-1}, initial pH=5.50, initial I=0.01 M. [$H_2C_2O_4$] mol L^{-1} : ① 0, ② $2.5x10^{-3}$, ③ $7.5x10^{-3}$, ④ $1.75x10^{-2}$, ⑤ $3.50x10^{-2}$, ⑥ $6.0x10^{-2}$, ⑦ $1.0x10^{-1}$ (with [Cu_{TS}]=$5.00x10^{-4}$ mol L^{-1}, without W).

Table 8. ESR spectral data for Cu(II) complexes with W and oxalic acid at 77K.†

[W]	$[H_2C_2O_4]$	$[Cu_{TS}]$	$g_\perp$	$g_\parallel$	$\lvert A_\parallel \rvert$
kg L^{-1}	mol L^{-1}	mol L^{-1}			cm^{-1}
4×10^{-3}	0	7.26×10^{-4}	2.043	2.314	1.84×10^{-2}
4×10^{-3}	2.5×10^{-3}	7.26×10^{-4}	2.043	2.314	1.84×10^{-2}
4×10^{-3}	7.5×10^{-3}	7.26×10^{-4}	2.038	2.311	1.89×10^{-2}
4×10^{-3}	1.75×10^{-2}	7.26×10^{-4}	2.038	2.310	1.89×10^{-2}
4×10^{-3}	3.50×10^{-2}	7.26×10^{-4}	2.038	2.320	1.79×10^{-2}
4×10^{-3}	6.00×10^{-2}	7.26×10^{-4}	2.051	--	--
0	1.0×10^{-1}	5.00×10^{-4}	2.051	--	--

-- unresolved

†Initial pH of W = 5.50, initial I of W = 0.01 M.

Cu(II)-W complex while the binding mechanism of the Cu(II)-WS_1 complex compares to the Cu(II)-SS complex.

Addition of pyridine (Py) to the Cu(II)-WSOF complex solutions resulted in complete displacement of the WSOF ligands by pyridine, forming $[Cu(Py)_4]^{2+}$. Complete displacement by pyridine was demonstrated with the appearance of the characteristic N hyperfine splitting on the ESR spectrum, and decreased $g_{\parallel}$ and increased $|A_{\parallel}|$ values (spectra not shown). Nearly 6 times the pyridine concentration ([Py] = 5.1×10^{-1} mol L^{-1}) was required to displace W from the Cu(II)-W complex solution ([W] = 4.00×10^{-3} kg L^{-1}, $[Cu_{TS}] = 7.26 \times 10^{-4}$ mol L^{-1}) than that ([Py] = 8.7×10^{-2} mol L^{-1}) from the Cu(II)-SS complex solution ([SS] = 4.00×10^{-3} kg L^{-1}, $[Cu_{TS}] = 8.19 \times 10^{-4}$ mol L^{-1}). This result may be attributed to the relatively stronger chelation in the Cu(II)-W complex.

CONCLUSIONS

The SS has a polyacidic nature while W has a relatively simple weak acidic nature. The SS may have at least three kinds of Cu^{2+} binding sites which are sensitive to pH changes. Oxygen donor ligands (probably, aromatic carboxyl groups) may be the major Cu(II) binding sites in the Cu(II)-W complex while sulfonate, carboxylate (probably aliphatic), and to a minor extent N-containing ligands, may be the major binding sites in the Cu(II)-SS complexes at pH 5. The Cu(II) complexation with N-containing groups increased as sludge was added to the soil.

Ligands of W may form relatively strong bonding to Cu^{2+} due to the extensive equatorial chelation while ligands of SS may form little

or no chelation with Cu^{2+}. In the latter case, each of the four in-plane ligands (e.g., COO^-, H_2O, Cl^-, etc.) may act independently as a monodentate ligand.

The Cu(II)-binding mechanism with WS_6 appears similar to the Cu(II)-W complex while the binding mechanism of the Cu(II)-WS_1 complex compares to the Cu(II)-SS complex. Consequently, bond strength of the Cu(II)-WSOF complexes followed the following order: Cu(II)-W > Cu(II)-WS_6 > Cu(II)-WS_1 > Cu(II)-SS. Based on the relative binding strength, Cu(II) added with sewage sludge should be more available to plants than indigenous soil Cu(II) chelated with soil organic matter. It appears that, with time, the Cu(II) added with the sewage sludge becomes chelated with stable soil organic constituents and availability decreases. The actual metal uptake by a plant, however, is also dependent upon the plant biochemistry and metal uptake mechanisms.

APPENDIX

The properties of ESR spectral parameters such as $g_{\parallel}$, $g_{\perp}$, and $A_{\parallel}$ have been previously summarized (Vänngård, 1972). If the molecule contains a single threefold or higher axis of symmetry (Z), then X and Y are equivalent. This is called axial symmetry and $g_{xx} = g_{yy} \neq g_{zz}$. The g factors are referred to as $g_{\parallel}$, the g factor parallel to the symmetry axis ($g_{\parallel} = g_{zz}$), $g_{\perp}$ for the g factor perpendicular to this axis ($g_{\perp} = g_{xx} = g_{yy}$). The g factors observed for Cu^{2+} are close to that of a free electron with spin motion only, that is 2.0023. The deviations from this value are caused by the spin-orbit coupling, which is an interaction between the magnetic moments associated with

the spin and orbital motions. This causes the unpaired electron to spend part of its time in other orbitals than $d_{x^2-y^2}$, and thereby some of the orbital motion is reintroduced to an extent that depends on the ratio of the spin-orbit coupling to the strength of the crystal field. Thus, approximately

$$g = 2.0023\ (1 + \gamma\lambda/\Delta) \qquad [A.1]$$

where λ is the spin-orbit coupling constant [-830 K (Kaiser)], γ is a constant (-4 for $g_{\parallel}$ and -1 for $g_{\perp}$), Δ is crystal-field splitting and ranges from 14 to 18 kK for Cu^{2+} in a tetragonal coordination with the unpaired electron in $d_{x^2-y^2}$.

Because of the combined effects of the crystal field splittings and the delocalization of the unpaired electron, there is an empirical correlation between the coordination atoms and the g factors. The largest g factors are obtained with oxygen as the ligand, smaller ones result from coordination to N, and still smaller from S coordination.

The sources of Cu hyperfine structure in Cu^{2+} compounds are illustrated by the following simplified theoretical expressions for the hyperfine coupling constant $A_{\parallel}$.

$$A_{\parallel} = P[-\kappa - \frac{4\alpha^2}{7} + (g_{\parallel}-2.0023) + \frac{3(g_{\perp}-2.0023)}{7}] \qquad [A.2]$$

where P is a constant (about 35 mK). The terms containing the factor α^2 arise from the dipole-dipole interaction between the magnetic moments associated with the spin motion of the electron and the nucleus. If the unpaired electron is delocalized to the neighboring atoms, this contribution is reduced as α^2 decreases from unity. The terms in κ arise from the Fermi contact interaction which has its

origin in a nonvanishing probability of finding the unpaired electron at the site of the nucleus and its numerical value is 0.3 to 0.4.

On the other hand, the ligand hyperfine coupling originates from dipole-dipole and Fermi contact interactions. The latter term is dominant for the N and is normally considered to arise from the delocalization of the unpaired electron into the s-part of a σ-bonding sp^2-hybrid on the nitrogen. For Cu^{2+} complexes with the unpaired electron in the $d_{x^2-y^2}$ orbital, couplings are expected to occur to the ligand atoms in the plane only and not to the axial ligands. When N splittings are detected by ESR, they frequently have a value of 10 to 15 G.

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COPPER (II) COMPLEXATION BY THE WATER SOLUBLE ORGANIC FRACTION OF A SEWAGE SLUDGE AMENDED SOIL

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ABSTRACT

Ratios of complexed metal (Mc) to free metal ions (M) at 296 K were measured in aqueous solutions of Cu(II) equilibrated with water soluble organic fractions (WSOF) extracted from an agricultural soil (W), a soil treated with sludge for 6 years (WS_6), a sludge-soil mixture incubated for one week (WS_1), and sewage sludge (SS). The [Mc]/[M] ratios were highest for complexes with SS, intermediate for WS_6 and WS_1, and lowest for W. The [Mc]/[M] ratio for the Cu(II)-SS complex at pH 5.5 was three times higher than that for the Cu(II)-W complex, probably due to the presence of more functional groups such as SO_3H and COOH. The Cu(II) appeared to form stronger bonds with WSOF than Mn(II) because the ESR spectral heights of free Mn^{2+} ion increased when Cu^{2+} was added to the Cu(II)- and Mn(II)-WSOF complex solution, indicating displacement of complexed Mn(II).

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INTRODUCTION

Stability constants can be used to predict the behavior of metal-organic complexes in soils. However, with soil organic matter, meaningful constants for metal complex formation are difficult to evaluate unless a detailed model for the structural chemistry of natural organic matter is known, including the type and number of complexes formed and their stoichiometry.

Schubert (1948) developed an ion-exchange method to determine the stability constants of complexes by measuring the distribution coefficient of a metal ion between a cation-exchange resin and the solution phase in the presence and absence of a complexing agent. The distribution coefficient λ_o of the metal ion, M^{n+}, between the resin phase and the solution phase in the absence of a complexing agent is defined by the equilibrium ratio

$$\lambda_o = \frac{[Mr]}{[M]} \qquad [1]$$

where [M] is the free metal ion concentration in solution and [Mr] is the number of moles of metal ion per unit weight of air-dried resin. The distribution coefficient λ in the presence of a complexing agent in solution is defined as the equilibrium ratio

$$\lambda = \frac{[Mr]}{[Mc] + [M]} \qquad [2]$$

where [Mc] is the concentration of the complexed metal ion in solution expressed in terms of gram atoms per unit volume. Combining Eqs. 1 and 2 gives

$$\frac{\lambda_o}{\lambda} - 1 = \frac{[Mc]}{[M]} \qquad [3]$$

Thus, the ratio of complexed metal to free metal ions in the solution for which λ is measured is given by $[(\lambda_o/\lambda)-1]$ regardless of the number or nature of the complexes formed in solution. This conditional ratio of complexed metal to free metal ions provides a useful parameter to compare the Cu(II) complexation with water soluble organic fractions extracted from soil or sludge amended soil (Lim et al., 1982a, b).

MATERIALS AND METHODS

Water soluble organic fractions (WSOF) from an agricultural soil (W), a soil treated with sludge for 6 years (WS_6), a sludge-soil mixture incubated for one week (WS_1), and sewage sludge (SS) from Portland, Oregon, were extracted and purified (Lim et al., 1982a). All discussion of the Cu(II) complexation will refer to only the WSOF of each sample.

Aliquots of the WSOF stock solution (2.5 mL), containing 4 g/L of WSOF were pipetted into 50 mL volumetric flasks and diluted to approximately 40 mL with distilled water. Five mL of 0.1 M $NaClO_4$ solution was added, followed by 3 mL of a Cu^{2+}-perchlorate solution (3.15×10^{-3} M Cu^{2+}). The pH of the solution was adjusted to either 4.0 or 5.5 with 0.1 M NaOH or 0.1 M $HClO_4$, after which the solution was diluted to 50 mL. Na-saturated Rexin 101 resin (1.000 g), 16-50 mesh (analytical grade) was weighed into 125 mL Erlenmeyer flasks. The solutions containing the Cu^{2+} ions and WSOF were transferred to these

flasks and shaken with the resin at 296 K for 5 hours. The exchange resin was removed by filtration. The filtrate was dried on a steam bath and digested with HNO_3-$HClO_4$ (Tan et al., 1971a, b). Each digest was diluted with distilled water to a volume of 50 mL and analyzed for Cu by atomic absorption spectroscopy.

Electron spin resonance (ESR) spectra for free Cu^{2+} and Mn^{2+} ions were obtained on a Varian E-4 spectrometer at 296 K (Lim et al., 1982b). Concentrations of free metal ions were assumed to be proportional to signal heights times line widths squared (Gamble et al., 1977). The complexed metal concentration (mol L^{-1}) was determined by difference between total metal concentration (M_{TS}) and free metal ion concentration. Cu(II) and Mn(II) complexes with WSOF were prepared by shaking the WSOF in $Cu(ClO_4)_2$ and $MnSO_4$ solutions, respectively.

RESULTS AND DISCUSSION

The [Mc]/[M] ratios for all Cu(II)-WSOF complexes increased as the pH values of the solutions increased from 4.0 to 5.5 indicating that more Cu(II) complexation occurred with decreased proton competition (Table 9). The [Mc]/[M] ratios were highest for Cu(II) complexes with SS, intermediate with WS_6 or WS_1, and lowest with W (Table 9). Since the ratios were determined at a constant WSOF concentration, it appears that the number of Cu(II) binding sites decreases as sludge added to the soil undergoes weathering. These results are consistent with the observations that the W has a lower content of functional groups (total acidity, COOH, phenolic OH) than SS (Lim et al., 1982a). Fulvic acid extracted from sewage sludge also complexed more Cu(II) than soil fulvic acid (Keech, 1979). Protein decomposition products,

Table 9. [Mc]/[M] ratios of Cu(II) complexes by water soluble organic fractions at 296 K.†

WSOF	[Mc]/[M] pH 4.0	[Mc]/[M] pH 5.5	$\frac{[Cu_{TS}]}{[WSOF]}$ mol kg^{-1}
W	0.28	0.49	0.94
WS_6	0.52	0.84	0.94
WS_1	0.74	1.04	0.94
SS	1.13	1.59	0.94

†$[Cu_{TS}]$ 1.89 x 10^{-4} mol L^{-1}, [WSOF] 2.00 x 10^{-4} kg L^{-1}, I = 0.01 M $NaClO_4$.

polysaccharides, and sulfonate groups in the water soluble fraction of a sludge amended soil were degraded rapidly and replaced by carboxyl group containing materials (Schaumberg et al., 1980). Condensation of humic materials during the weathering process may cause the decrease in metal complexing sites.

The Cu(II) complexation of the WSOF samples increased with the total Cu(II) concentration (Fig. 9). However, a maximum Cu(II) binding capacity for W and WS_6 appears to be attained somewhere near the value of 0.1 mol Cu(II)/kg W or WS_6 (Fig. 9). Apparent maximum Cu(II) binding capacities were not reached for WS_1 or SS. These results are also consistent with the data for the functional group analysis (Lim et al., 1982a).

When the Cu_T concentration in a given solution of Cu(II)- and Mn(II)-WSOF complexes was increased with the addition of Cu^{2+} solution, the heights of six hyperfine Mn^{2+} ESR spectra increased, indicating that Cu(II) displaced the complexed Mn(II) (Fig. 10). On the other hand, when the Mn_T concentration in a given solution of Cu(II)- and Mn(II)-WSOF complexes increased with addition of a stock Mn^{2+} solution, the height of the Cu^{2+} ion spectrum was not changed, indicating that the Cu(II) complex was stronger than the Mn(II) complex.

It has generally been assumed that the stronger retention of Cu(II) on the humic material is due to the formation of an inner-sphere complex while Mn(II) forms a weaker outer-sphere complex. However, the inner- and outer-sphere complexes in metal-humic complexes have not been distinguished by direct spectroscopic evidence. The Cu^{2+} has greater polarizability than Mn^{2+}. Therefore, Cu^{2+} may have the stronger bonding strength on the humic material.

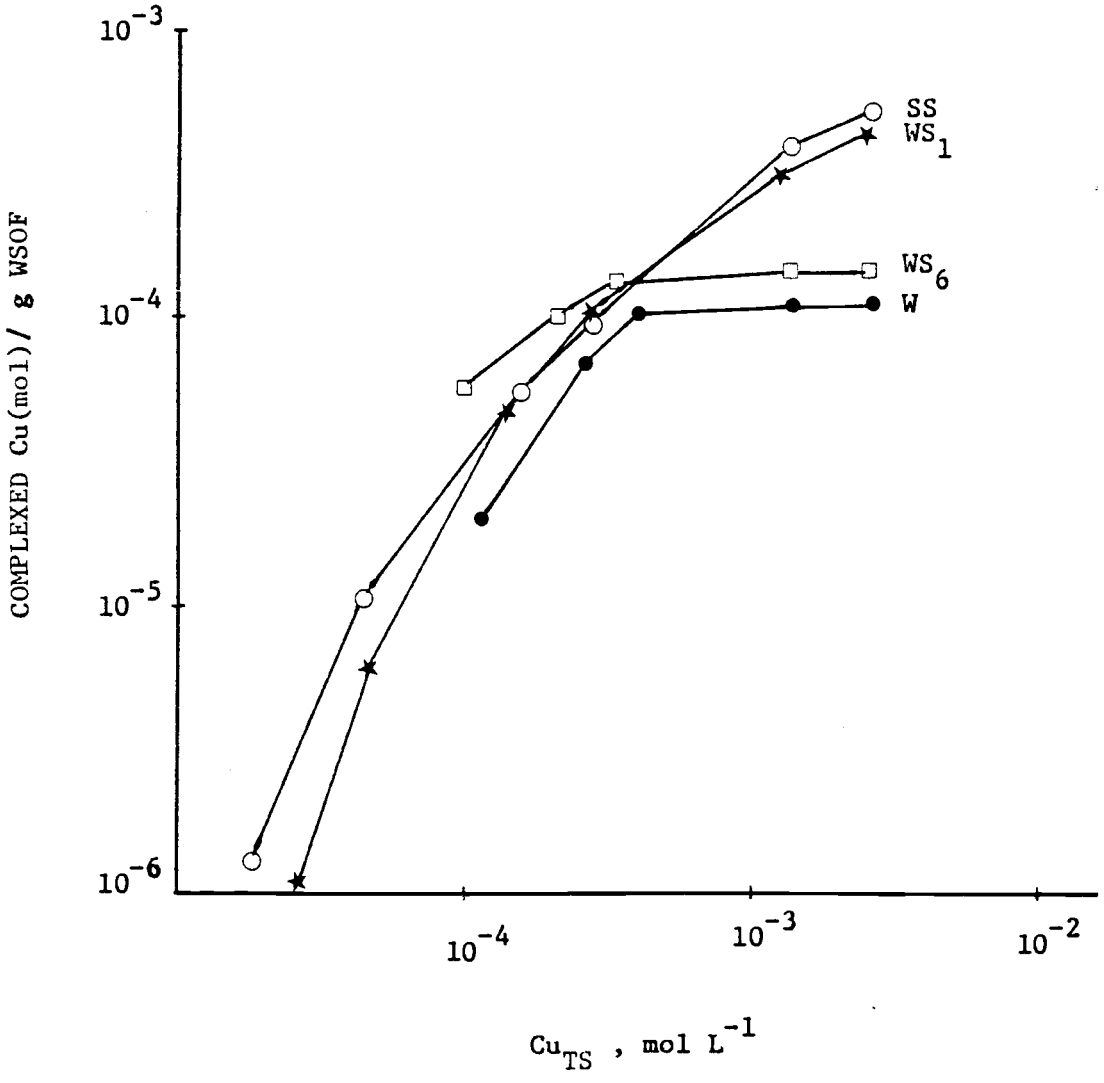

Figure 9. Cu(II) complexation with water soluble organic fractions at 296 K. [WSOF]=1.0x10^{-3} kg L^{-1}, I=0.01 M, pH=5.50 .

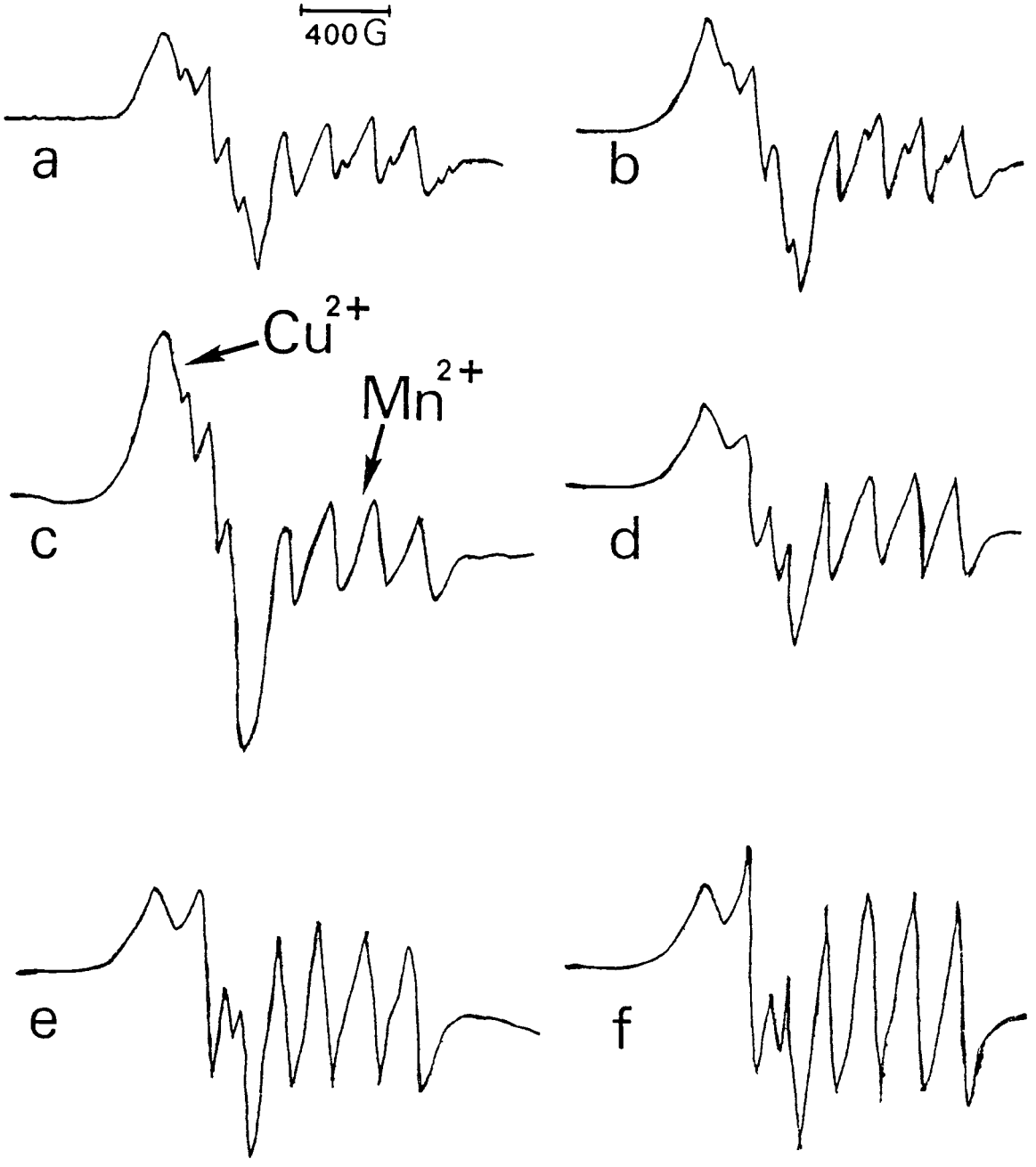

Figure 10. ESR spectra of Cu(II)- and Mn(II)-W complexes at 296 K.
[W]=1.0×10^{-3} kg L^{-1}, I = 0.01 M, pH = 5.50.
$[Cu_{TS}]$ mol L^{-1}: a, 1.19×10^{-4} ; b, 1.47×10^{-4} ; c, 2.55×10^{-4}; d,e,f, 1.19×10^{-4}.
$[Mn_{TS}]$ mol L^{-1}: a,b,c, 1.68×10^{-6} ; d, 3.04×10^{-6} ; e, 4.40×10^{-6} ; f, 7.12×10^{-6}.

CONCLUSIONS

The ratio of complexed metal (Mc) to free metal ions (M) in water soluble organic fractions increased as sewage sludge was added to the soil. A maximum Cu(II) binding capacity for W and WS_6 was attained near the value of 0.1 mol Cu(II)/kg WSOF while it was not reached for WS_1 or SS. Consequently, an amendment of sewage sludge to the soil appears to increase the Cu(II) complexing capacity of a soil; however, the bonding strength between the SS and Cu(II) is weaker than the bond between W and Cu(II).

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CONCLUSIONS

Sewage sludge added to a soil appears to degrade and humify during the aging time (1-6 years) as indicated by the similarity in analytical properties between WS_6 and W. The SS has higher N and COOH contents, and a lower S content than fulvic acid extracted from sewage sludge. The W has a lower C and H content, and a much lower total acidity and COOH content than the "model" soil fulvic acid. Therefore, SS appears to contain more proteinaceous material with a higher carboxyl group content than sewage sludge fulvic acid, and W may have less O-containing functional groups, suggesting that the WSOF may be quite different from fulvic acid extracted with an alkali solution.

Ligands of W may form relatively strong bonds with Cu(II) due to extensive equatorial chelation while ligands of SS may form little or no chelation with Cu(II). Based on the relative binding strength, Cu(II) added with sewage sludge should be more available to plants than indigenous soil Cu(II) chelated with soil organic matter. With time, the Cu(II) added with the sewage sludge would become chelated with stable soil organic constituents and availability decrease. The actual metal uptake by a plant, however, is also dependent upon the plant biochemistry and metal uptake mechanisms.

The ratio of complexed metal to free metal ions in water soluble organic fractions increased as sewage sludge was added to the soil. Consequently, an amendment of sewage sludge to the soil appears to increase the Cu(II) complexing capacity of a soil; however, the bonding strength between the SS and Cu(II) is weaker than the bond between W and Cu(II).

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