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Complexation of Copper(II) in Organic Soils and in Dissolved Organic Matter − EXAFS Evidence for Chelate Ring Structures

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Associations with functional groups of natural organic matter (NOM) are of great importance for bioavailability, toxicity, and mobility of trace metals in soils and waters. In this study, the coordination chemistry of copper, Cu(II), in organic soils and dissolved organic matter (DOM) from soils and streams was investigated by extended X-ray absorption fine structure (EXAFS) spectroscopy. In both soil organic matter (SOM) and DOM (990−11 000 μg Cu g^{-1} dry weight, pH 2.8−6.3), Cu(II) was coordinated by 4 oxygen/nitrogen (O/N) atoms at a distance of 1.92−1.95 Å in the first coordination shell. These four atoms are positioned in the equatorial plane of a Jahn−Teller distorted octahedron. In samples with a pH of 4.8−6.3, a second coordination shell with 2.0−3.8 C atoms was located at a distance of 2.76−2.86 Å. A significant improvement (19−39%) of the fit was obtained by including a third coordination shell with 2.0−3.8 O/C atoms involved in single scattering at an average distance of 3.69 Å and multiple scattering at an average distance of 4.19 Å. Our results provide evidence for inner-sphere complexation of Cu(II) in NOM and suggest that Cu(II) is complexed by either one or two five-membered chelate rings involving possible combinations of amino, carboxyl, or carbonyl functional groups. Ion activity measurements showed that less than 0.2% of total Cu was in the form of free Cu^{2+} in our samples at pH 4.8−6.3.

Introduction

It is generally accepted that concentrations of specific metal species are of higher significance than total metal concentrations for the bioavailability and toxicity of trace metals in soils and waters. For copper (Cu), the free metal ion (Cu^{2+}) and soluble inorganic complexes are assumed to be the most toxic and bioavailable forms (e.g., refs *1* and *2*). Thus, dissolved organic matter (DOM) has been shown to reduce both toxicity and bioavailability of Cu to fish (*3*, *4*). Of special importance for the speciation are chemical interactions with surfaces, and Cu(II) is known to bind to functional groups of natural organic matter (NOM), clay minerals, and iron, manganese, and aluminum oxyhydroxides (*5*−*8*). NOM in soils and waters consists of a complex mixture of organic substances containing a variety of different organic functional groups such as carboxyls (−COOH), phenols (−OH), thiols (−SH), and amines (−NH$_2$). All of these groups may play an important role in the complexation of Cu (*9*).

Associations of Cu(II) with NOM and its functional groups are not well described, especially at low metal concentrations. There are no reports where Cu(II) associated to organic soils have been studied by extended X-ray absorption fine structure (EXAFS) spectroscopy, and only few studies cover the coordination chemistry of Cu(II) in humic substances and humic acids (*7*, *10*−*12*). These studies were conducted at comparatively high Cu/organic C mole ratios (>0.02) and showed that the first coordination shell of Cu(II) displays a Jahn−Teller distorted geometry, with four O atoms in the equatorial plane and two O atoms in axial positions. On the basis of a qualitative interpretation of the X-ray absorption near edge structure (XANES) region, Frenkel et al. (*13*) argued that N-containing functional groups are likely to be engaged in the complexation of Cu(II) at low Cu/C ratios (<0.005) in aquatic humic substances. To our knowledge, there are only two studies in which EXAFS data for the second shell contributions in NOM have been reported. Xia et al. (*10*) investigated the binding environment of Cu(II) in a soil humic substance and reported four C atoms in the second shell at a distance of 3.11−3.16 Å, whereas Lee et al. (*12*) found 2.9 C atoms at a distance of 2.88 Å in an aquatic humic acid. Besides providing information for distinguishing between outer- and inner-sphere complexes, a more detailed quantification of second and possible third shell contributions is needed to better understand the biogeochemistry of Cu on a molecular scale in soils, sediments, and waters.

Copper is a chalcophilic metal that forms insoluble sulfide minerals in reducing environments (*6*). EXAFS studies of other chalcophilic metals such as Hg, CH$_3$Hg, Cd, and Zn have shown that these metals bind to reduced organic S (Org-S$_{red}$) in NOM (*14*−*17*). Thus, it is possible that also Cu(II) binds to reduced S groups, at least at low Cu/Org-S$_{red}$ ratios. Numerous studies have revealed that Cu(II) are stabilized by five-membered ring structures in many well-defined organic molecules involving mainly carboxyl groups or a combination of carboxyl and amino groups (*18*−*20*). In this paper, we present EXAFS results for the coordination chemistry (first, second, and third shell contributions) of Cu(II) in organic soils and in DOM from soils and a stream at Cu concentrations of 1000−11 000 μg g^{-1}, corresponding to Cu/organic C and Cu/Org-S$_{red}$ ratios of 0.0004−0.0058 and 0.03−2.21, respectively.

Material and Methods

Sampling, Sample Preparation, and Chemical Analysis. Soil organic matter (SOM) samples were collected at four different locations. A subalpine fen peat (SFP) dominated by *Carex spp.* was sampled at Ifjord, northern Norway, situated within 5 km from the Atlantic Ocean (70°,5′ N, 27°,1′ E). A boreal forest peat soil (BFP), covered by *Picea abies* and *Sphagnum* and *Polytricum* mosses, and a histic organic (O) horizon (POH) and an organic-rich spodic (B$_{hs}$, illuvial) horizon (PBH) from a Spodosol (*21*), covered by *P. abies*, *Vaccinium* shrubs, and *Hylocomnium splendens* and *Pleurozium schreberi* mosses, were sampled at Svartberget Research station, Vindeln, Sweden (64°,14′ N, 19°,46′ E). A cultivated *Phragmites* fen peat (AFP) was sampled at Majnegården, Falköping, in southwest Sweden (58°,0′ N, 14°,5′ E). Finally, a peat soil (Slagnäs) highly contaminated by Cu, Zn, As, and Pb was sampled at a marshalling yard in Slagnäs, Sweden (65°,6′ N,

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TABLE 1. Chemical Composition of Samples Prepared for EXAFS Analysis: Reduced Organic Sulfur (Org-S_{red}), Organic Carbon (Org-C), and Total Cu (Cu_{tot})

sample	[Org-C] (g kg^{-1})	[Org-S_{red}] (g kg^{-1})	[Cu_{tot}] (μg g^{-1})	pH	[Cu/Org-S_{red}] (mol/mol)	[Cu/Org-C] (mol/mol)	% free Cu^{2+} [f]	% $\sum$ $Cu(OH)_n$ [g]
thiol resin			15 000	6.0	0.10		<5	<0.1
carboxyl resin			15 000	7.2		0.026	<0.1 bd[h]	<0.1
Slagnäs[a]	338	nd[c]	6200	4.1		0.0035	9	<0.1
BFP1[a]	493	2.5[d]	10 100	2.8	2.04	0.0039	~50	<0.1
BFP2[a]	493	2.5[d]	11 000	5.3	2.21	0.0042	<0.1	<0.1
SFP1[a]	410	15[d]	990	5.4	0.03	0.0005	<0.2 bd[h]	<0.1
SFP2[a]	410	15[d]	2800	5.2	0.09	0.0013	<0.2	<0.1
AFP[a]	427	5.4[d]	1000	4.8	0.10	0.0004	<0.2 bd[h]	<0.1
POH[a]	565	1.8[e]	2600	6.0	0.71	0.0009	<0.1 bd[h]	<0.1
DOH[b]	431	0.8[e]	2600	6.3	1.64	0.0011	<0.1 bd[h]	<0.1
DBH1[b]	329	nd[c]	2500	6.1		0.0015	<0.1 bd[h]	<0.1
DBH2[b]	329	nd[c]	10 000	6.1		0.0058	<0.1 bd[h]	<0.1
SRN[b]	525	1.8[e]	3600	5.5	1.00	0.0013	<0.1 bd[h]	<0.1

[a] SOM. [b] DOM. [c] nd = not determined. [d] Determined by XANES. [e] Determined by XPS. [f] ($[Cu^{2+}]/[Cu_{tot}]$) × 100. [g] ($([CuOH^+] + [Cu(OH)_2{}^0{}_{(aq)}])/[Cu_{tot}]$) × 100. [h] bd = below the detection limit.

18°,2′ E). The samples were treated following protocols for a clean sampling procedure, sealed in double plastic bags, and stored at 4 °C. All samples, except the one from Slagnäs, were freeze-dried (Edwards Modulyo 4K Freeze-dryer) and homogenized by a tungsten carbide ball mill (Retsch, S2, Germany). The sample from Slagnäs was only homogenized by grinding in a mortar.

Three different types of dissolved organic matter (DOM) were also used: Suwannee River NOM (SRN) (processed by the International Humic Substance Society, IHSS) and soil DOM extracts from the POH sample (DOH) and from the PBH sample (DBH). Suwannee River NOM was collected from the Suwannee River near Fargo, GA and concentrated by reverse osmosis. The DOM extracts from the Spodosol O and B_{hs} horizons were obtained using a modified method of Adams and Byrne (*22*). A mass of 50−100 g of soil and 6−18 g of technical grade ion-exchange resin in Na form (Chelex 20, Bio-Rad) were weighed into 250 mL centrifuge bottles (Nalgene), and 75−150 mL of Milli-Q water was added. The soil and ion-exchange resin were left to equilibrate for 1−16 h. After equilibration, the bottles were centrifuged at 10750 RCF (Beckman J2-21M/E model) for 5 min and the supernatant was collected by decantation. Additional amounts of Milli-Q water (75−50 mL) were added to the mixture of soil and ion-exchange resin, and the extraction procedure was repeated 4−5 times for the DOH sample and 2−3 times for the DBH sample. The sum of all DOM extracts was finally separated from soil particles by a prewashed filter paper (Munktell 3, pore size <10 μm, STORA, Sweden). The pH was determined to be 6.5 and 6.6 in the DOH and DBH extracts, respectively. Finally, the DOM extracts were freeze-dried (Edwards Modulyo 4K Freeze-dryer) and stored in darkness.

Total sulfur in SOM and DOM samples was determined on a LECO sulfur analyzer (LECO Corp., MI). Reduced organic S was determined either as the sum of sulfur species showing absorption peak maxima in the energy range 2472−2474 eV, using S K-edge XANES, according to the procedure of Xia et al. (*23*) and Skyllberg et al. (*24*) or as the sum of sulfur species with binding energy peak maxima in the range 162−164 eV, using X-ray photoelectron spectroscopy (XPS), in accordance with Urban et al. (*25*). Soil organic carbon was determined by combustion on an elemental analyzer (Perkin-Elmer, 2400 CHN). An MP 220 or an MP 20 pH meter (Mettler, Toledo) was used for pH measurements.

For the EXAFS experiments and measurements of copper ion (Cu^{2+}) activity, 100 mg of the SOM samples or 50 mg of the DOM samples was weighed into 2 mL Eppendorf tubes with $Cu(NO_3)_2$ dissolved in Milli-Q water to yield Cu concentrations ranging from 990 to 11 000 μg g^{-1} on a dry mass basis. To adjust the pH (2.8−6.3) and set the ionic strength, KOH (0.5 M) and $NaNO_3$ (10 mM) solutions were added. The suspensions were shaken and then left to equilibrate for 24−72 h. Subsequently, the tubes were opened and the suspensions were left to evaporate at room temperature in a fume hood for 24−48 h. No precaution was taken to exclude $CO_{2(g)}$ from the suspensions because no sample was oversaturated with respect to formation of $CuCO_{3(s)}$ and contributions from the dissolved species $CuCO_{3(aq)} + CuHCO_3{}^+{}_{(aq)}$ were negligible at pH 2.8−6.3. The total equilibrium time applied was more than 1 week for all samples. Before EXAFS analysis, the remaining wet paste was mounted in Teflon sample holders and sealed with Kapton tape (CHR-Furon). The ionic strength in samples analyzed by EXAFS was calculated to be in the range of 20−100 mM, after correcting for losses of water during evaporation.

Compounds obtained by addition of Cu to ion-exchange resins were considered to be relevant as reference compounds for Cu-NOM complexes. However, bond angles and coordination numbers may differ somewhat from Cu-NOM complexes due to physical constraints in the resins. A thiol resin (Amberlite GT-73, Rohm and Hass) was used to prepare a Cu-SR model compound and a carboxylic resin (BioRex 70, Bio-Rad) to prepare a Cu-OOCR model compound. The ion-exchange resins were protonated in 1 M HCl for 30 min, filtered through a filter paper (Munktell 3), and then rinsed with Milli-Q water. A mass of 500−600 mg of protonated ion-exchange resin (corresponding to a dry mass of 200 mg) was weighed into 2 mL Eppendorf tubes and $Cu(NO_3)_2$ dissolved in Milli-Q water was added, yielding Cu concentrations of 15 000 μg g^{-1} on a dry mass basis. This corresponds to a Cu/SR ratio of 0.10 and a Cu/OOCR ratio of 0.026. To adjust the pH to 6, KOH (0.5 M) was added and the tubes with the suspensions were shaken and then left to equilibrate for 24−72 h. The equilibrated suspensions were drained through filter paper (Munktell 3), and the resin was collected in Eppendorf tubes. Before EXAFS analysis, the resins were ground in a mortar, mounted into the sample holders, and sealed with Kapton tape. Selected chemical data for the samples prepared are shown in Table 1.

Ion Activity Measurements with an Ion Selective Electrode (ISE). The Cu^{2+} activity in equilibrium with adsorbed Cu was determined in all resins, SOM, and DOM samples analyzed by EXAFS. Measurements were made in suspensions (1.65−2.0 mL of solution to 0.03−0.1 g of dry mass of sample) with 7 mM $NaNO_3$, using an Orion 96-29 ionplus cupric electrode in conjunction with a Mettler Toledo SevenMulti in millivolt (mV) mode. The ionic strength was calculated to be in the range of 8−22 mM and the detection limit of the ISE was 7.8×10^{-7} M. On the basis of the measurements, the percentage of free Cu^{2+} in solution and hydrolysis species $CuOH^{+}_{(aq)} + Cu(OH)_2{}^0_{(aq)}$ was calculated for all samples (Table 1). Calculations were also made to investigate if any sample was oversaturated with respect to formation of the solid phases, $CuO_{(s)}$ and $Cu(OH)_{2(s)}$. Stability constants (at $I = 0$, 25 °C) from Smith and Martell (*26*) and Stumm and Morgan (*1*) were used in the calculations. Activities of Cu^{2+} were calculated by the Davies equation (*1*).

EXAFS Data Collection and Analysis. Data collection was performed at MAX-lab (Lund University, Sweden) at the superconducting multi-pole wiggler beam line i811 with 1.5 GeV beam energy and 100−200 mA electron current. Cu K-edge (8.979 keV) spectra were recorded at room temperature at ambient atmospheric pressure in fluorescence mode using a double crystal monochromator (Si [111]). The energy was calibrated by measuring a Cu foil in transmission mode. The fluorescence signal was detected with a Lytle detector filled with Ar, and a Ni filter was placed between the detector and the sample to reduce unwanted scattering and fluorescence contributions. Samples were positioned at 45° to the incident beam. The monochromator was detuned 15−25% to remove higher order harmonics. EXAFS data (I_f/I_0 against energy) were collected at an exposure time of 2−4 s for each data point with 5 eV steps from 200 eV before the edge and 1 eV steps from the edge and up to 800 eV after the edge. Each EXAFS spectrum represents the average of 1−6 scans, each scan taking 30 or 60 min to collect depending on exposure time.

As a first approach, EXAFS data for all SOM and DOM samples were qualitatively compared in *k*-space and *R*-space with two different model compounds, Cu(II)-*N*-(phosphonomethyl)glycine (Cu(II)-PMG) and $Cu(NO_3)_2$ in water solution ($Cu(H_2O)_6{}^{2+}$). Models having C in the second coordination shell were fitted to data for samples that were similar to Cu(II)-PMG, and a model with first shell O atoms only was fitted to data for samples that could not be distinguished from $Cu(H_2O)_6{}^{2+}$. For reduction and fitting of EXAFS data, WinXAS97 (*27*) was used. From each averaged spectrum, a polynomial pre-edge function was subtracted and the data were normalized. Above the absorption edge, a cubic spline fit was used to remove the background and the data were k^3-weighted to enhance data at higher *k*-values. Theoretical EXAFS amplitudes and phase functions of mixed models for Cu−O and Cu−C associations generated by FEFF 7.0 (*28*) were fitted to the experimental spectra. These models were based on structural parameters from well-defined organic and inorganic compounds (*11*, *18*−*20*, *29*). The models were fitted to EXAFS data in *k*-space over the interval 3.1−12.3 Å^{-1}. During the fitting procedure, the edge energy (ΔE_0) was allowed to vary, but kept internally constant for all shells in a sample. To reduce the number of fitting parameters, the Debye−Waller factor (σ^2) was constrained between 0.004 and 0.01 for second and third shell contributions and the coordination number (CN) for the first shell was fixed to 4.0. Coordination numbers for the second and third shells were correlated. The amplitude reduction factor ($S_o{}^2$) was determined to be 0.7, based on the fit of the carboxyl resin (Cu-OOCR). This value was used when different models were fitted to data for all NOM samples.

Different models were tested to describe the EXAFS data for samples showing a contribution from C in the second coordination shell. As a first approach, CNs for the second shell were allowed to float. In the next step, a model with third shell contributions, based on a five-membered chelate ring structure, was tested. This model is supported by EXAFS data and fits for the coordination chemistry of Cu(II) in PMG (*18*) and glutamate (*19*). The model used for the final fits to EXAFS data included single scattering (SS) paths for first, second, and third shell contributions as well as a multiple scattering (MS) path for the third shell contribution. EXAFS data for all NOM samples were also examined for possible occurrence of Cu in higher coordination shells, which would indicate formation of multinuclear complexes or precipitation of $Cu(OH)_{2(s)}$. Furthermore, the XANES region (8.97−9.0 keV) was qualitatively evaluated for all NOM samples to possibly distinguish between O and N contributions in the first coordination shell (cf., ref *13*).

Results and Discussion

ISE Measurements. The ISE measurements showed that free Cu^{2+} was below 0.2% of total Cu in samples with pH ranging from 4.8 to 6.3 and Cu concentrations of 990−11 000 μg g^{-1} (Table 1). For the model compounds, free Cu^{2+} in solution constituted less than 5% of total Cu in the thiol resin and less than 0.1% in the carboxyl resin. At a Cu concentration of approximately 10 000 μg g^{-1} and a pH of 2.8, approximately 50% of total Cu was in the form of free dissolved Cu^{2+} in the BFP1 sample. In the Slagnäs sample (6200 μg Cu g^{-1}), 9% was free Cu^{2+} in solution despite a pH of 4.1 (Table 1). This is probably due to the high concentrations of Zn (∼20 000 μg g^{-1}) and Pb (∼4000 μg g^{-1}) in this sample, which leads to a competition for binding sites between the metals. From this, we can conclude that free hydrated Cu^{2+} ions only contributed to the EXAFS result in sample BFP1 and Slagnäs. Furthermore, equilibrium calculations showed that the dissolved hydrolysis species $CuOH^{+}_{(aq)}$ and $Cu(OH)_2{}^0_{(aq)}$ constituted less than 0.1% of total Cu in all samples (Table 1) and that all samples were undersaturated with respect to formation of $CuO_{(s)}$ and $Cu(OH)_{2(s)}$.

EXAFS Results for the First Coordination Shell. Table 2 and Figure 1 show the final fitting results in *k*-space for the carboxylic resin and all NOM samples. Corresponding Fourier transforms are illustrated in Figure 3 (Supporting Information). The first coordination shell is composed of 4 O/N atoms at a distance of 1.92−1.95 Å in all NOM samples and in the carboxylic resin. These four atoms are most likely positioned in the equatorial plane of a Jahn−Teller distorted octahedron, where the contribution to the EXAFS signal from the more distant axial oxygens is expected to be small (*18*). There was no significant improvement in the fits of the first coordination shell when axial O/N were included, as compared to a model with only equatorial O/N. The O/N distances (1.92−1.95 Å) in our NOM samples are in accordance with data for Cu(II) coordinated by 4 equatorial O, or a combination of 4 O/N, in a variety of different inorganic and organic molecules (Table 3). They are also in accordance with data presented for soil humic acid, 1.94 Å (*7*), soil humic substance, 1.93−1.94 Å (*10*), and an aquatic humic acid, 1.94 Å (*12*).

There are no indications for S associations in any of our NOM samples, not even in the SFP1 and AFP samples where the Cu/Org-S_{red} molar ratios are very low (0.03 and 0.10, respectively). Earlier EXAFS studies have shown S contributions in the SFP sample for CH_3Hg and Cd at approximately the same or higher metal/Org-S_{red} ratios (*15*, *16*).

EXAFS Results for Higher Coordination Shells. The EXAFS spectra of the carboxylic resin and the two SOM samples with the highest concentrations of free Cu^{2+} (BFP1 and Slagnäs) showed small differences as compared to the spectrum for $Cu(H_2O)_6{}^{2+}$ (Figure 1). Therefore, no result for second shell contributions will be presented for these samples.

All other NOM samples have a more or less pronounced shoulder at the second oscillation in *k*-space that is lacking in $Cu(H_2O)_6{}^{2+}$ (Figure 1). This shoulder is also very obvious in the model compound Cu(II)-PMG. In PMG, Cu(II) is positioned at the center of a Jahn−Teller distorted octahedron with three donor groups (amine, carboxylate, phosphonate) taking part in the binding, forming two five-membered

TABLE 2. Final Fits to EXAFS Data in *k*-Space for Cu(II) in the Carboxylic Resin and in the Different NOM Samples[a]

	1st shell Cu−O/N$_{eq}$ (SS)			second shell Cu−C (SS)			third shell Cu−O/C (SS)			Cu−C−O/C (MS)				
sample	CN	*R* (Å)	$\sigma^2 \times 10^3$ (Å^2)	CN	*R* (Å)	$\sigma^2 \times 10^3$ (Å^2)	CN	*R* (Å)	$\sigma^2 \times 10^3$ (Å^2)	CN	*R* (Å)	$\sigma^2 \times 10^3$ (Å^2)	ΔE_0 (eV)	merit of fit (%)[d]
carboxyl resin	4	1.95	4.3										−1.9	5.4
Slagnäs[b]	4	1.94	3.1										−2.3	7.2
BFP1[b]	4	1.95	3.5										−1.4	6.4
BFP2[b]	4	1.94	3.8	2.5	2.83	10	2.5	3.71	8.5	5.0	4.19	9.2	−1.0	3.1
SFP1[b]	4	1.94	4.1	3.1	2.83	10	3.1	3.71	7.8	6.2	4.23	5.7	−0.5	8.5
SFP2[b]	4	1.92	3.6	2.0	2.82	6.1	2.0	3.67	7.3	4.0	4.14	4	−2.7	5.5
AFP[b]	4	1.92	4.5	2.2	2.76	4	2.2	3.71	4	4.4	4.16	7.3	−2.2	8.5
POH[b]	4	1.93	3.8	2.7	2.82	6.9	2.7	3.67	9.9	5.4	4.20	7.8	−2.2	4.8
DOH[c]	4	1.94	5.9	3.2	2.86	9.1	3.2	3.70	10	6.4	4.20	9.6	−0.9	6.2
DBH1[c]	4	1.94	5.8	3.8	2.86	8.6	3.8	3.70	9.1	7.6	4.24	8.9	−0.6	7.8
DBH2[c]	4	1.92	4.1	3.1	2.82	7.2	3.1	3.66	8.0	6.2	4.15	9.5	−2.1	3.2
SRN[c]	4	1.94	3.7	3.5	2.84	10	3.5	3.69	8.5	7.0	4.23	10	−1.0	3.1

[a] The amplitude reduction factor (S_0^2) was determined to be 0.7: soil organic matter (SOM), dissolved organic matter (DOM), edge energy (ΔE_0), coordination number (*CN*), bond distance (*R*), Debye−Waller factor (σ^2), single scattering (SS), and multiple scattering (MS). [b] SOM. [c] DOM. [d] The merit of fit (%) is defined as $((\Sigma(\chi_{exp} - \chi_{fit})^2)/(\Sigma(\chi_{exp})^2)) \times 100$; χ_{exp} and χ_{fit} represent experimental and fitted data points, respectively.

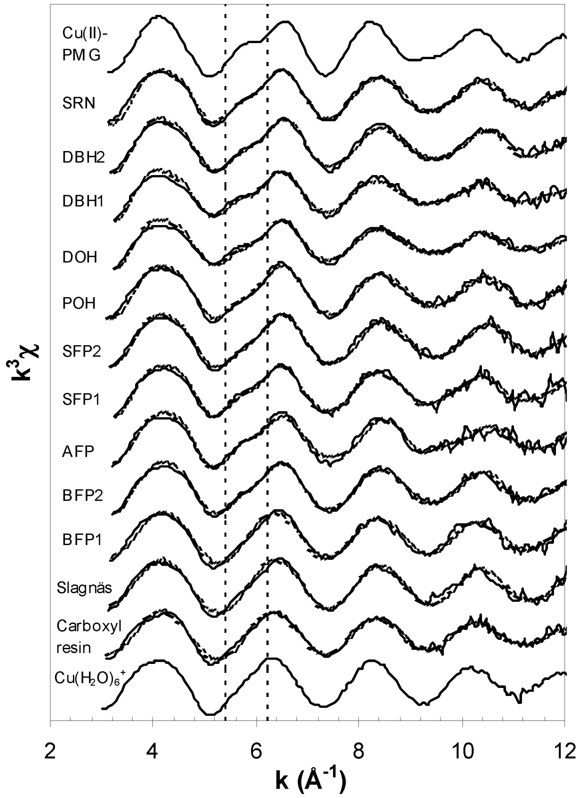

FIGURE 1. *k*^{3}-weighted EXAFS spectra for NOM samples, the carboxylic resin, Cu(II)-PMG (*18*), and $Cu(H_2O)_6^{2+}$. Solid lines represent experimental spectra, and dashed lines represent spectra derived from fitting parameters (Table 2). The two vertical dashed lines indicate a more or less distinctive shoulder reflecting third shell contributions from O/C atoms.

chelate rings (*18*). Second shell SS from C and P in the five-membered ring and SS and MS from O/C atoms in the third shell give rise to the shoulder in *k*-space (*18*), which appears in the Fourier transforms as a prominent peak at ∼2.4 Å (not corrected for phase shift) and as a weak feature between 3.2 and 3.7 Å, respectively (Figure 3, Supporting Information). In Cu(II) complexed by glutamate, second shell SS from C and third shell SS and MS from O/C give rise to a feature similar to that for Cu(II)-PMG (*19*). The significant contribution from the third shell is due to a close to linear arrangement of Cu−C−O, resulting in a relatively strong MS.

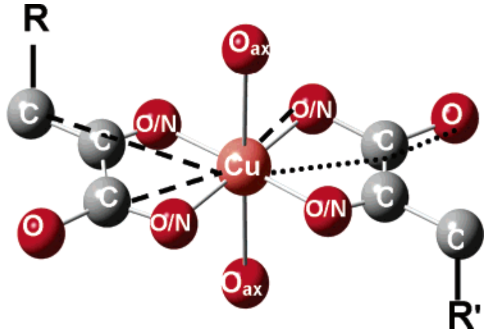

FIGURE 2. Average structure for Cu(II) in NOM. The dashed lines indicate the three single scattering (SS) paths, and the dotted line indicates the multiple scattering (MS) path, identified in the EXAFS analysis. The more distant axial oxygens (O$_{ax}$) are not included in the final fits to the EXAFS data. R represents carbon chains.

A model similar to Cu(II) complexed by glutamate (*19*) gave the best fit to data for the NOM samples with a significant second shell contribution (Table 2). Distances between Cu(II) and second and third shell C and O atoms are also all well in agreement with compounds in which Cu(II) forms five-membered ring structures (Table 3). As compared to a model with only SS paths in the first and second shell, an inclusion of SS+MS paths for O/C atoms in the third shell resulted in fits that were on average 28% better, with a range of 19−39% improvement (Table 4, Supporting Information). Much of this improvement is due to the MS contribution, as illustrated in Figure 4 (Supporting Information). When CNs in second and third coordination shells were correlated and allowed to float, 2.0−3.8 C atoms were encountered at distance of 2.76−2.86 Å and 2.0−3.8 O/C atoms at an average distance of 3.69 Å. The close to linear alignment of Cu−C−O gave rise to a relatively strong MS at an average distance of 4.19 Å, with a CN correlated to be 2 × CN of the second shell (Table 2). The final fitting results strongly suggest that Cu(II) is complexed by O/N functional groups in NOM, forming a structure involving one or two five-membered chelate rings (Figure 2). Thus, the CN of 2.0−3.1 in SOM and 3.1−3.8 in DOM suggests that the Cu-complexes are mainly represented by one ring in SOM and mainly by two rings in DOM. This is reasonable, because DOM in average consists of smaller and more flexible (less sterically hindered) organic molecules.

With EXAFS, it is not possible to distinguish between O and N atoms, which is well-known from studies of well-defined organic molecules where Cu(II) form five-membered

TABLE 3. Bond Distances (*R*) for Cu(II) in Water Solution ($Cu(H_2O)_6{}^{2+}$), Tenorite ($CuO_{(s)}$), *N*-(Phosphono-methyl)glycine (Cu-PMG), Glutamate (Cu-GLU), and Bis(*S*-methyl-L-cysteine) (Cu-SMC)

	1st shell Cu−O/N_{eq}		Cu−O_{ax}		second shell Cu−C		third shell Cu−O/C		MS[b] Cu−C−O/C		
compound	CN	*R* (Å)	CN	*R* (Å)	CN	*R* (Å)	CN	*R* (Å)	CN	*R* (Å)	ref
$Cu(H_2O)_6{}^{2+}$	4	1.97	2	2.24							11
CuO(s)	4	1.95−1.96	2	2.78							29
Cu-PMG[a]	4	1.96			3	2.81	2	3.72	2	4.08	18
Cu-GLU	4	1.94			4.1	2.80	2.0	3.73	2.3	4.14	19
Cu-SMC	4	1.94/2.00[c]	2	2.72	4	2.77[d]			4	4.16[d]	20

[a] One P atom is located at a distance of 3.01 Å. [b] Multiple scattering path. [c] Two O atoms at 1.94 Å and 2 N atoms at 2.00 Å. [d] Average distances calculated from interatomic distances and bond angles.

ring structures involving functional groups such as carboxyls, carbonyls, and amines (*18−20*). Thus, the structure in Figure 2 should be seen as an average of several possible combinations of O- and N-containing functional groups. A carbon chain (R) is attached to each ring to show that the ring structure likely is a part of a larger molecule. This molecule in turn may show a very large heterogeneity. Hence, the ring structure may be associated with humic substances or with different kinds of biomolecules. At the comparatively low Cu concentrations used in this study, it is possible that small organic molecules contribute significantly to the results.

As compared to the two EXAFS studies (*10*, *12*) that report data for the second shell in NOM, our determined Cu−C distance of 2.76−2.86 Å is well in accordance with the Cu−C distance of 2.88 Å reported by Lee et al. (*12*) for an aquatic humic acid. Xia et al. (*10*) reported a longer Cu−C distance of 3.11−3.16 in a soil humic substance. They used samples with Cu/organic C ratios higher than 0.065, and there is no visible shoulder at the second oscillation in *k*-space in their data.

Data isolated from higher coordination shells in *R*-space and then back-filtered to *k*-space showed no indications of heavy backscatters in any of our NOM samples, ruling out polynuclear Cu complexes or Cu precipitates. The two SOM samples (BFP1 and Slagnäs) showing EXAFS spectra similar to $Cu(H_2O)_6{}^{2+}$ have low pH, 4.1 and 2.8, respectively. However, as indicated by the ISE measurements, a significant fraction of Cu is complexed by NOM in these samples (50% in BFP1 and 91% in the Slagnäs sample). The absence of second shell contributions in these samples, and in the carboxylic resin (<0.1% free Cu^{2+}), suggests a formation of outer-sphere complexes, that is, Cu adsorbed in hydrated form ($Cu(H_2O)_6{}^{+}$), and/or a very distorted average geometry. Hence, there is a possibility of a mixture of outer- and inner-sphere complexes and free Cu^{2+} in these samples. The Slagnäs samples have high concentrations of other metals, approximately 20 000 μg Zn g^{-1} and 4000 μg Pb g^{-1}, that may compete with Cu for binding sites, enhancing the formation of weaker outer-sphere Cu−NOM complexes and free Cu^{2+} in solution.

Qualitative Interpretation of the XANES Region. Frenkel et al. (*13*) used the XANES region to determine Cu binding sites in aquatic humic substances. They found a pre-edge feature in the XAS spectra and their first derivatives (between 8.976 and 8.980 keV) at low Cu/C ratios, which they interpreted as involvement from N-containing functional groups in the first coordination shell. This interpretation was based on theoretical FEFF calculations as well as observations of similar features in model compounds. For our DOH and DBH1 samples, a similar pre-edge feature was observed between 8.975 and 8.980 keV (Figure 5, Supporting Information). This pre-edge feature was lacking in all other NOM samples as well as in the carboxylic resin. Still, as Frenkel et al. (*13*) clearly state, more detailed theoretical ab initio calculations and XANES studies of well-defined systems are needed to elucidate this matter. Thus, at this point, we cannot rule out a significant N contribution in the first coordination shell in NOM.

Environmental Implications. At pH 4.8−6.3 and metal concentrations of 990−11 000 μg Cu g^{-1}, Cu(II) form inner-sphere complexes consisting of one to two five-membered chelate rings in NOM. Furthermore, ISE measurements showed that less than 0.2% of total Cu was free Cu^{2+} in solution. This suggests that Cu is strongly complexed by NOM functional groups also in heavily polluted organic rich soils and surface waters. Accordingly, DOM most definitely plays an important role in determining toxicity, mobility, and bioavailability of Cu in these systems. The binding structures presented for Cu(II) in SOM and DOM are important starting points for further in-depth studies of the relationship between speciation and bioavailability of Cu in soils and waters.

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Supporting Information Available

The merit of fit for two different models fitted to EXAFS data in *k*-space for all NOM samples (Table 4), Fourier transformed radial structure functions for all NOM samples, the carboxylic resin, Cu(II)-PMG, and $Cu(H_2O)_6{}^{2+}$ (Figure 3), k^3-weighted EXAFS spectra, experimental spectra and spectra derived from fitting parameters for two different models, for the DBH1 and AFP samples (Figure 4) and first derivatives of the X-ray absorption spectra in the Cu K-edge XANES region for the DOH and DBH1 samples and the carboxylic resin (Figure 5). This material is available free of charge via the Internet at http://pubs.acs.org.

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