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Identification of Copper Binding Sites in Soil Organic Matter through Chemical Modifications and ^{13}C CP-MAS NMR Spectroscopy

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Metal binding to an organic peat soil was probed by paramagnetic doping with copper, chemical modifications of the organic matter in the soil, and ^{13}C CP-MAS NMR spin lattice relaxation rate measurements. Carboxyl and hydroxyl functional groups were determined to be most significant in copper uptake by the unmodified soil. Esterification and acetylation of the soil showed that metal binding by carbohydrate structures occurs independently of other functional groups and may even induce a pseudochelation phenomenon. Sorption isotherms corroborate the importance of carbohydrate structures in metal binding. These results suggest that environmental modeling of metal binding and retention in soils should incorporate estimates of the distributions of all functional groups in the soil organic matter (e.g. aliphatic, carbohydrate, phenolic, carboxyl) and their relative binding strengths.

Introduction

Metals in trace amounts are ubiquitous in natural systems and function as an important source of mineral nutrients for higher plants. However, at elevated levels, these metals can become toxic and pose serious health risks. Humic substances, naturally occurring organic matter found in terrestrial and aquatic environments, play a key role in the transport and bioavailability of these metal ions in the environment. The affinity of humics for metals can be attributed to their highly functionalized nature which comprises various oxygen-containing moieties, of which carboxyl and phenol are thought to be the most influential in metal ion binding.

Nonliving biomass has recently received a great deal of attention as a remediation tool for the removal of harmful metals from the environment. However, to effectively use biomass for metal extraction, an understanding of the nature of the chemical binding process is vital. Many studies have reported the significance of biomass functionality as well as solution pH on metal ion uptake through chemical modifications (*1*−*7*). For instance, Gardea-Torresday et al. showed a decrease in Cu(II) binding after esterification of carboxyl groups on peat moss and its humic fractions (*5*) as well as on an algal species (*6*). The role of amino and hydroxyl groups collectively as well as carboxyl groups in the metal binding of Cr(IV) was investigated through chemical modifications using *Rhizopus nigricans* (*7*). Again, a decrease in metal uptake was observed after the blockage of these functional groups. Conversely, the addition of carboxyl and amino groups to various types of biomass was shown to increase metal sorption capacity (*4, 7*).

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Since its introduction by Opella and Frey in 1979 (*8*), solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy using the Cross Polarization (CP) and Magic Angle Spinning (MAS) with dipolar dephasing has proven to be one of the most valuable techniques for studying intact, natural organic materials such as coals (*9*) and humic substances (*10*). This technique also provides a means to follow the binding of paramagnetic metal cations through the various "compartments" of intact biomass, particularly very complex and heterogeneous materials such as natural soil organic matter. Paramagnetic cations can act as probes of metal binding sites in soil organic matter due to their effects on spin lattice relaxation rates of chemically distinct and spatially separated domains where the metal binding occurs. When intimate contact between paramagnetic centers such as Cu^{2+} and Fe^{3+} and particular carbon nuclei exists, more efficient nuclear relaxation occurs with a corresponding reduction in the spin lattice relaxation rates ($T_{1\rho}H$ and the more sensitive T_1H) during a CP-MAS NMR experiment. The net effect is often selective signal loss and peak broadening for those carbon species involved in the metal binding (*11*).

Spin lattice relaxation in the rotating frame, $T_{1\rho}H$, relates to the time taken for the excited proton spins to lose energy and coherence to their surroundings in the presence of a radio frequency field which is applied during the cross polarization process. $T_{1\rho}H$ relaxation rates are typically less than 10 ms for geochemical materials, and thus structural heterogeneity can only be probed on a scale of less than 30 nm (*12*). Spin lattice relaxation, T_1H, relates to the time needed for excited proton spins to lose energy to their surroundings and return to their equilibrium state in the presence of an external magnetic field which is supplied by the NMR magnet. T_1H rates are usually greater than 50 to 75 ms, so heterogeneity can be probed on scales ranging from 30 to 100 nm (*13*).

Spin lattice relaxation rate measurements by solid-state ^{13}C CP-MAS NMR have contributed significantly to our understanding of metal−natural organic matter interactions (*14*−*23*). For example, Peffer et al. (*17*) used Fe^{3+} to follow metal binding in model sludge components based on spin lattice relaxation rates and found a reduction in $T_{1\rho}H$ and T_1H values for the more hydrophilic components in iron doped samples compared to those relaxation rates for hydrophilic components in unamended sludge. These reductions were particularly noticeable for protein and carbohydrate groups, suggesting that metal binding was associated with the more acidic exchangeable proton sites. Preston et al. (*18*) also showed a possible preferential localization of copper in the carbohydrate or hydrophilic portions of four organic soils. Paramagnetic cation doping was used to probe lignin-carbohydrate associations in wood pulp as well (*19*). Smernik and Oades (*11*) looked at the effects of added paramagnetic and nonparamagnetic ions on the relaxation rate constants for a deashed soil and were able to distinguish differences between the paramagnetic and nonparamagnetic ions as well as differences between paramagnetic ions themselves (e.g. Mn^{2+}, Fe^{3+}, and Cu^{2+} were unique). They suggested uronic acid-type structures were involved in metal binding. Cook and Langford (*22, 23*) used ^{13}C CP-MAS NMR relaxation rate measurements to conclude that aliphatic and carbohydrate hydroxyl groups were primarily responsible for copper binding and not phenols as had been suggested.

This work was undertaken to build on these previous studies by identifying the important metal binding sites in an organic peat soil using copper doping and chemical modifications combined with relaxation rate measurements by ^{13}C CP-MAS NMR. This approach allows for changes in

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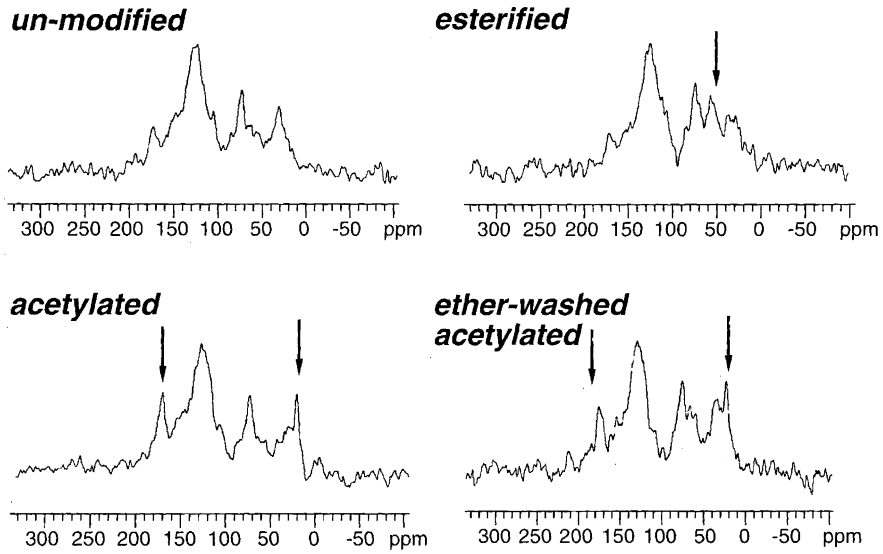

FIGURE 1. ^{13}C CP-MAS NMR spectra of peat soil before and after chemical modifications. Arrows indicate functional groups that were altered by the specific modification.

metal binding to be followed directly through spin dynamics as a result of the blockage of specific sites in soil organic matter. The goal was to provide a more comprehensive understanding of the role specific functional groups play in metal−humate interactions.

Materials and Methods

Soil. The soil used was a commercially available peat which was sieved (<74 μm) and deashed 5 × 1 h according to Schilling et al. (*24*) using hydrofluoric acid. Briefly, 15 g of the sieved peat was placed into a 125 mL polyethylene bottle (Nalgene, Rochester, NY) and treated with 30 mL of a 2% HF/BF_3 solution. Treatments were stirred for 1 h and filtered through P5 qualitative paper (Fisher Scientific, Fairlawn, NJ). The peat was subsequently washed five times with pure Millipore water (18MΩ) (Millipore, Bedford, MA), and the treatment was repeated 4 × 1 h. The soils were then homogenized and oven-dried at 60 °C overnight and stored in glass vials until further analysis.

Copper analysis was performed on 0.2 M HNO_3 digests by flame atomic absorption spectroscopy (Varian, Palo Alto, CA) based on a previously developed digestion procedure (*24*) with detection at 324.8 nm. Briefly, 100 mg of peat was placed into a 15 mL Teflon vial (Savillex, Minnetonka, MN) along with 2 mL of 8 M HNO_3 (Fisher, Fairlawn, NJ) and heated at 60 °C for 30 min. Upon cooling, 1 mL of 48% HF was added and heated at 60 °C for 1 h, and the acid solution was then evaporated to dryness. The resulting residue was allowed to cool, and then 2 mL of 6 M HCL was added and evaporated to dryness. Finally, 20 mL of 1 M HNO_3 was added in 10 mL increments, filtered through a 0.45 μm syringe filter (Whatman, UK), and diluted to 100 mL. Digests were stored at 4 °C in polypropylene bottles (Nalgene, Rochester, NY) until analysis.

Soil samples were doped using the procedure of Smernik et al. (*8*). Briefly, 0.4 g of deashed unmodified/modified peat were mixed with 10 mL of 0.5 M $CuCl_2 \cdot 2H_2O$ for 1 h. Samples were subsequently centrifuged, and the copper solution was decanted. Ten mL of pure Millipore water (18 MΩ) (Millipore, Bedford, MA) was added, and the process was repeated to remove residual Cu^{2+} ions. The washing step was carried out twice more, after which the sample was oven-dried at 60 °C and stored in glass vials until further analysis.

Chemical Modifications. Esterification was carried out using 4.25 g of deashed peat to which 125 mL of methanol (Purge and Trap Grade, Fisher Scientific, Fairlawn, NJ) was added with 1.5 mL of concentrated HCl (Fisher Scientific, Fairalwn, NJ) and refluxed for 24 h. After removal of the excess methanol, the peat was washed with cold Millipore water and allowed to dry at 60 °C. Acetylation was carried out using 4.0 g of deashed peat to which 125 mL of acetic anhydride (A.C.S grade, Fisher Scientific, Fairlawn, NJ) was added with 1.5 mL of glacial acetic acid and stirred at 25 °C for 18 h, followed by heating at 90 °C for 6 h. Once again, after removal of the excess acetic anhydride, the peat was washed with cold Millipore water and allowed to dry at 60 °C.

Qualitative comparisons of soil spectra verified that these chemical modifications did not substantially alter the relative abundances of functional groups in the organic matter; in all cases, the only changes in the spectra were increases in the functional groups chemically bonded to the material (Figure 1). In addition, sorption experiments were carried out on the esterified material after the attached esters were released through a base hydrolysis. Although the NaOH solution did change to a light brown color, the concentration of metal in the supernatant for the sorption experiments of the hydrolyzed sample was not substantially different than that of the original, unmodified peat sample. If leaching of any metal binding groups did occur, one would expect the metal concentration in the supernatant to be higher than that of the unmodified sample. We therefore believe that, even though some material might have been leached in the base hydrolysis, the overall chemical characteristics of the organic matter were not altered.

^{13}C CP-MAS NMR Spectroscopy. Solid-state ^{13}C CP-MAS NMR spectra of unamended or Cu^{2+} amended unmodified, esterified, and acetylated peat samples were obtained at a ^{13}C frequency of 75.5 MHz on a Bruker DMX 300 MHz spectrometer. Samples were packed in a 4 mm rotor and spun at the magic angle at 10 kHz. A sweep width of 33 kHz and a sampling of 1 K data points with 200 Hz of line broadening were used throughout. Chemical shifts were externally referenced to the 43 ppm resonance of glycine. Variable contact time experiments (*25*) were performed using a 4.0 μs 90° pulse width and a recycle delay of 2 s with contact times ranging from 50 μs to 4.5 ms. Inversion recovery experiments also were run under the same conditions according to Sullivan et al. (*26*) incorporating a 2 s recycle delay and recovery delays ranging from 100 μs to 2 s along with a 1.5 ms contact time. One to four thousand transients were collected for each sample. $T_{1\rho}H$ values were calculated from the signal intensities of the major resonances in the

peat sample from variable contact time experiments according to Smernik et al. (*20*). A computer fit of the signal intensities as a function of contact time for the decay portion of the cross polarization curve gave $T_{1\rho}H$. For T_1H estimations, a computer fit of signal intensities as a function of recovery delay was carried out (*21*). All fits gave T_1H and $T_{1\rho}H$ correlation values greater than 0.96 and 0.80, respectively.

Dipolar depashing experiments (*27*) were also performed using a 60 μs dephasing delay between cross polarization and data acquisition and a 180° refocusing pulse along with a 2 s recycle delay in order to probe the protonation of carbon in the peat soil.

Sorption Isotherms. Fifty milligrams of unmodified, esterified, or acetylated peat was mixed with 10 mL of $CuCl_2 \cdot 2H_2O$ in 15 mL Falcon tubes (Becton Dickinson, Franklin Lakes, NJ) such that copper concentrations ranged from 5 to 50 ppm (pH = 4). After 20 h, by which time the apparent equilibrium of copper uptake was reached based on initial kinetics experiments, samples were centrifuged, and aliquots of supernatant were removed to be analyzed for copper content. Copper uptake by the peat was calculated from the difference between initial and final copper concentrations in the supernatant. Metal uptake from solution by the Falcon tubes was deemed negligible based on blanks (no peat) carried through the kinetics experiments. All isotherms were run in triplicate and fitted to the Freundlich model. This model can be expressed as $x/m = K_F{*}C_e^{1/n}$ where x/m is the amount of copper(II) sorbed per gram of peat (expressed as μg/g), K_F is the Freundlich sorption coefficient, C_e is the concentration of copper(II) remaining in solution (μg/mL), and 1/n is a constant which describes sorption nonlinearity. Values of K_F and 1/n were obtained through plots of log x/n versus log C_e for each sample.

Results and Discussion

^{13}C CP-MAS NMR Spectroscopy. ^{13}C CP-MAS NMR allows for the characterization of the structural properties of whole, heterogeneous material such as soil organic matter, whose chemistry is highly dependent on decomposition processes. Figure 1 depicts the spectral changes resulting from the chemical modification of the peat sample. The unmodified sample (Figure 1a) shows a peat that is extremely aromatic with low lignin content, thus suggesting that this soil is highly mature in nature. The spectrum of the esterified peat shows a distinct resonance at 57 ppm, which is not present in the spectrum of the unmodified sample. This peak is assigned to the methoxy carbon, which results from the conversion of carboxyl groups to ester groups in the soil. The peak is not the result of methanol sorption to the sample, which would show a distinct resonance at 49 ppm due to noncovalent binding. However, it can be seen that some aliphatic material was removed from the peat as evidenced by a reduction of the 32 ppm resonance, probably from polymethylene material.

The spectrum of the acetylated peat shows a marked increase in the resonances at 17 and 173 ppm compared to the unmodified sample. These peaks result from the methyl and carbonyl resonances, respectively, due to the conversion of mainly hydroxyl groups to esters. However, acetylation can convert both aliphatic and aromatic hydroxyl groups to esters. To determine the extent to which aromatic hydroxyls (i.e. phenolic groups) were derivatized, dipolar dephasing NMR experiments were carried out. In the dipolar dephasing experiment, the proton channel is turned off for a fixed period of time (60 μs in our experiments) before data acquisition. This disrupts cross polarization and allows ^{13}C nuclei to relax at a rate that depends on the number of protons both directly bonded to the carbon nuclei and on adjacent carbon atoms. Since aromatic carbon nuclei bonded to hydroxyl groups have no directly attached protons, they relax at a relatively slow rate and are thus still observed after an appropriate dipolar dephasing delay period.

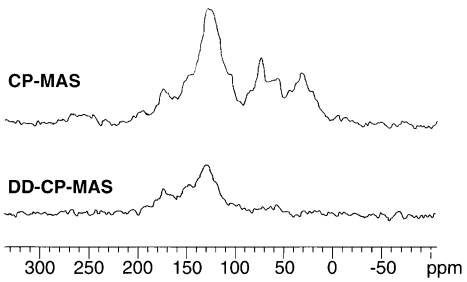

FIGURE 2. Normal CP-MAS ^{13}C NMR spectrum of unmodified soil (top) and dipolar dephased spectrum (bottom) after 60 μs dephasing period.

Figure 2 shows the dipolar dephased spectrum of the unmodified peat. The phenol signal at around 150 ppm is relatively small in the dephased spectrum compared to the resonances in the 50−100 ppm region in the normal CP-MAS spectrum. The peaks in the 50−100 ppm region are most likely associated with hydroxyl groups of cellulose material, although some ether and amine functionality may be represented as well. Given the small phenol signal in the dephased spectrum and low nitrogen content of this soil, we conclude that most of the acetylation probably takes place at aliphatic hydroxyl sites.

To determine if the increased peaks resulted from acetic anhydride contamination, the acetylated peat sample was stirred for 24 h in diethyl ether, in which acetic anhydride is soluble, washed, and then allowed to dry. As is evident in Figure 1, the peaks in the aliphatic and O-alkyl region increase in intensity, whereas the peak at 173 ppm remains at constant intensity. This increase in the aliphatic/O-alkyl region directly results from ether contamination. However, if acetic anhydride were sorbed by the peat, the peak at 173 ppm would be expected to decrease in intensity after an ether wash. Thus, we believe that the increased intensities at 17 and 173 ppm in the acetylated peat spectrum are indeed due to acetylation of aliphatic hydroxyl groups and not residual acetic anhydride.

Spin Dynamics and Metal Binding. Spin dynamics of the unmodified and modified peat can be utilized to follow the effect on metal binding sites resulting from chemical modification. Table 1 shows the T_1H and $T_{1\rho}H$ values for each type of peat sample. The unmodified soil exhibits proton spin relaxation rates that are in close agreement with other studies using naturally occurring material (*19*). The relative changes (as % reduction) in T_1H and $T_{1\rho}H$ due to Cu^{2+} doping were then determined in order to assess metal binding to the major functional groups of the sample. An enhancement of paramagnetically induced T_1H relaxation in the unmodified peat is evident in the carboxyl (173 ppm) and O-alkyl (74 ppm), and to a lesser extent in the aromatic region (127 ppm), perhaps due to minor copper binding to phenol groups, where paramagnetic-induced relaxation may be partially felt by nearby aromatic carbon moieties or the presence of aromatic structures in the vicinity of carboxyl/carbohydrate copper binding. $T_{1\rho}H$ values also indicate a similar trend in the carboxyl and O-alkyl peaks. A $T_{1\rho}H$ value could not be determined for the aromatic peak for any unamended or copper doped sample due to a lack of signal decay at longer contact times, most likely the result of a slow cross polarization of these condensed aromatic structures because of their remoteness to proton-rich moieties.

Upon esterification, a dramatic reduction in metal binding to carboxyl groups is evident by the much lower effect of copper doping on T_1H and $T_{1\rho}H$ relaxation rate of the 173 ppm resonance when compared to the unmodified SOM.

TABLE 1. Spin Lattice Relaxation Rates (ms) for (a) T_1H and (b) $T_{1\rho}H$

sample	173 ppm	127 ppm	74 ppm	32 ppm
(a) T_1H				
unmodified/unamended peat	38.5	31.7	75	52.6
unmodified Cu^{2+} amended peat	0.42	19.1	15.8	56
% change	−99	−40	−79	+6
esterified unamended peat	32.1	42.8	81.8	53.1
esterified Cu^{2+} amended peat	17.2	15.1	19.4	22.7
% change	−46	−65	−76	−57
acetylated unamended peat (ms)	46.7	34.5	55.9	65.7
acetylated Cu^{2+} amended peat (ms)	15.8	18.5	30.5	55.6
% change	−66	−46	−45	−15
(b) $T_{1\rho}H$				
unmodified/unamended peat	10.4		11.1	5.7
unmodified Cu^{2+} amended peat	3.4		9.2	4.1
% change	−67		−17	−28
esterified unamended peat	10.9		8.1	6.9
esterified Cu^{2+} amended peat	11.4		7.3	4.2
% change	+5		−10	−39
acetylated unamended peat	24.5		22.6	5.7
acetylated Cu^{2+} amended peat	10.6		10.4	4
% change	−57		−54	−30

Ester and amide groups, with resonances that overlap with the carboxyl peak at 173 ppm, are thought to coordinate metal ions much more weakly than carboxyl groups. The data in Table 1 confirm that these groups are not involved to any great extent in metal binding, since it would be expected that the relaxation behavior of the 173 ppm resonance in esterified sample would be more similar to the relaxation of this peak in the unesterified SOM. However, an increase in T_1H relaxation was observed at the 127 ppm resonance, perhaps once again due to metal binding to phenol groups, where paramagnetic-induced relaxation may be partially felt by nearby aromatic carbon moieties, since carboxyl groups are no longer available. There is also an unexpectedly similar trend in the aliphatic region. This selective binding of copper is also reflected in the $T_{1\rho}H$ values of the 173 and 32 ppm peaks in this esterified sample. Relaxation of the O-alkyl resonance at 74 ppm, on the other hand, does not change dramatically, indicating that copper binding at this site is unaffected by the esterification of carboxyl groups. Some reports have suggested that the simultaneous reduction in carboxyl and O-alkyl T_1H and $T_{1\rho}H$ values are the result of uronic acid type structures (*11, 18*). Our data, however, suggest that binding to carboxyl and O-alkyl sites are independent and uronic acid structures are probably not involved. There are data to support this conclusion: cellulose, for example, has been shown to bind appreciable amounts of copper, even at low pH (*28*−*30*). Leenheer and co-workers in an elegant series of experiments were able to isolate the principal copper-binding fractions of aquatic fulvic acids (*31*). Structural analyses by ^{13}C CP-MAS NMR indicated that short-chain dibasic acids were particularly important metal binding sites, but the majority of the fulvic acid molecule was involved, resulting in polydentate binding sites capable of both inner- and outer-sphere binding.

The acetylated sample shows a diminished T_1H relaxation in the O-alkyl region due directly to the blockage of the hydroxyl sites with ester groups. The changes of the spin dynamics in the carboxyl, aliphatic, and O-alkyl resonances in the undoped, acetylated sample from that of the undoped, unmodified peat results from the actual modification of the cellulose material in the soil. As can be seen from Cu^{2+} doping of the acetylated peat, T_1H relaxation increases in the carboxyl region by 66% but not to the degree observed in the unmodified sample, where relaxation increased by 99%. However, aromatic and aliphatic relaxation times after doping return to unmodified levels with relaxation changes of around 40−45% and less than around 15%, respectively, after acetylation. A lack of change of aliphatic and aromatic relaxation rates for the copper doped acetylated sample compared to the copper doped unmodified peat suggests that carboxyl and aliphatic/aromatic carbon moieties are spatially separated. This result suggests that, upon esterification, some type of interaction occurs between carbohydrate and aromatic/aliphatic moieties. Cook et al. (*23*) reported similar behavior with O-alkyl and aliphatic resonances upon copper doping of a fulvic acid and suggested that the aliphatic side chains were in the vicinity of complexation sites, or metal ion complexation induced a change in the conformation of the organic structure, which brings the aliphatics in closer proximity to the metal binding sites (pseudochelation). In our work, esterifying the carboxyl sites and shifting the copper binding to aromatic and/or aliphatic sites makes this pseudochelation effect resulting from interactions between these non-carboxyl groups even more plausible. It should be noted that hydrogen bonding between aliphatics and water molecules complexed to the metal may also explain this phenomenon. $T_{1\rho}H$ values drastically changed after acetylation for the 173 and 74 ppm resonances due to the incorporation of ester groups on the cellulose material.

TABLE 2. Copper Content of Doped Peat Samples

sample	copper content (% mass)
unmodified/unamended peat	trace
unmodified Cu^{2+} amended peat	1.06
esterified unamended peat	trace
esterified Cu^{2+} amended peat	1.09
acetylated unamended peat	trace
acetylated Cu^{2+} amended peat	1.05

It has often been suggested that the hydroxyl and carboxyl groups on the aromatic rings comprise the major metal binding sites in natural organic matter. However, these results indicate that aliphatic hydroxyl and carboxyl groups are the preferential binding sites in this peat soil. If these groups were incorporated on an aromatic ring, it would be expected that an accompanying increase in relaxation would occur at 127 ppm after copper doping due to the paramagnetic-induced relaxation being felt by aromatic carbon moieties in the vicinity of copper binding. Phenols have been shown to bind metals only at higher metal loading (*23*). Other studies have suggested that aliphatic and carbohydrate hydroxyl groups comprise the majority of weakly acidic protons and are more involved in metal binding than phenolic groups (*22*). Our results support those observations. It must be noted that these results are specific to our particular peat sample and that a less decomposed sample containing more phenol groups may exhibit different behavior.

Table 2 includes the copper concentrations of each doped soil sample. Regardless of the chemical modification, the same amount of metal is contained in each sample. Since this material certainly contains multidentate sites, it seems that the copper is being "redistributed" throughout the sample, either by direct binding (i.e. from carboxyl/O-alkyl to phenol/O-alkyl) or by some sort of pseudochelation as described by Underdown et al. (*32*). This pseudochelation may involve a cooperative effect in which the natural organic molecule self-associates to stabilize the bridged complex.

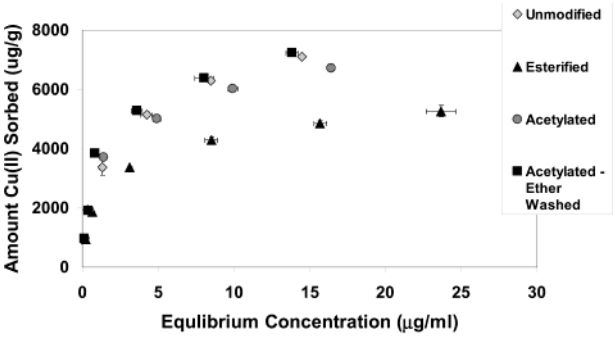

FIGURE 3. Unmodified and chemically modified peat sorption isotherms.

TABLE 3. Freundlich Isotherm Fitting Parameters

sample	K_F (μg/g)/(μg/mL)$^{1/n}$	1/*n*
unmodified peat	2700 ± 82	0.40 ± 0.01
esterified peat	1930 ± 97	0.35 ± 0.03
acetylated peat	2470 ± 23	0.40 ± 0.01
ether-washed acetylated peat	2850 ± 166	0.41 ± 0.01

The observed decrease in the multidentate nature of the metal binding (i.e. carboxyl/O-alkyl to O-alkyl/aliphatic) upon esterification may allow more freedom for organic matter structural conformational changes which can bring these metal binding sites in closer proximity to aliphatic and aromatic carbon containing structures. The similarity in metal contents of the three samples also indicates that all available metal binding sites are saturated.

Sorption Isotherms. Sorption isotherms were constructed to probe metal binding as a function of chemical modification at lower copper concentrations. The isotherms are plotted in Figure 3, with accompanying Freundlich constants listed in Table 3. As expected, blockage of carboxyl groups gives the greatest reduction in the Freundlich sorption coefficient, with a decrease of ~28%. This confirms the importance of carboxyl groups in metal binding. Acetylation of the sample gives a K_F reduction of around 9%, suggesting hydroxyl groups are involved to some degree in metal binding, but not to such an extent as carboxyl groups. At lower metal loading conditions only the more energetic sites (i.e. carboxyl) will be involved in binding, and thus a greater decrease is seen after esterification than after acetylation. A portion of the remaining metal binding capacity of the soil organic matter after both chemical modifications may be due, in part, to a failure to completely block all hydroxyl/carboxyl groups. This preferential behavior was not evident in the NMR doped samples (Table 2) because of the much higher copper loading conditions, and thus all favorable sites are involved in binding. The sorption of ether in the diethyl ether washed acetylated sample that was inferred from the ^{13}C NMR spectrum was confirmed by the sorption isotherm. An increase in metal uptake at least to and possibly above the unmodified sample is likely due to the additional oxygen functionality imparted to the soil. If the esterified sample were contaminated with methanol or the acetylated sample with acetic anhydride, it would be expected that similar behavior would be observed in the sorption data for these two samples. The fact that this behavior was not observed confirms that true chemical modification of the sample has indeed occurred. Also, to determine whether a decrease in copper binding was due to chemical or thermal degradation of the peat sample during the modifications, and not the result of functional group blockage, sorption experiments were performed after base hydrolysis with NaOH (pH = 12) of the esterified peat which converts ester groups back to carboxyl groups. The copper binding ability of the subsequent peat was completely restored (data not shown), suggesting the esterification was indeed responsible for the decrease in copper uptake.

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