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Environ. Sci. Technol., 2009, 43 (7), 2417-2424 • Publication Date (Web): 05 March 2009

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Molecular Rigidity and Diffusivity of Al$^{3+}$ and Ca$^{2+}$ Humates As Revealed by NMR Spectroscopy

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Received October 4, 2008. Revised manuscript received January 27, 2009. Accepted February 10, 2009.

NMR techniques were applied to follow changes in molecular rigidity and diffusion of complexes formed between a humic acid (HA) and either aluminum or calcium ions, added in amounts ranging from 0.05% to 1% of HA carboxylic acidity. Spin–lattice relaxation time in the rotating frame ($T_1\text{(H)}$) and diffusion coefficients ($D$) of humic–metal complexes were obtained from $^{13}$C cross-polarization magic angle spinning (CP-MAS), and $^1$H-diffusion order spectroscopy (DOSY) spectra, respectively. Molecular rigidity of humic complexes increased significantly with metal addition throughout the full carbon spectral region, being more pronounced for triple-charged Al than for double-charged Ca. However, $T_1\text{(H)}$ values of spectral intervals suggested that molecular rigidity increase was generally in the following order: aliphatic C > aromatic/double bonds C > carboxyl C. Concomitantly, DOSY spectra showed that addition of both Al and Ca decreased substantially the diffusivity of humic alkyl components and increased that of aromatic and hydroxylalkyl components, thereby indicating that complexation induced a molecular-size increase in the former and a decrease in the latter. These results suggest that saturated and unsaturated long-chain alkanoic acids in HA were preferentially involved in metal complexation with Al and Ca, with consequent increase of conformational rigidity and molecular size of humic hydrophobic domains. Conversely, more hydrophilic or mobile humic components appeared relatively less affected by the molecular and intermolecular rearrangements induced in HA by complexation with metals. Such NMR approach appears thus liable to evaluate the response to metal complexation of specific chemical entities present in the bulk HA and provides a further insight in the molecular architecture of humic–metal complexes.

Introduction

Humic substances (HS) represent the largest biospheric carbon pool, and their complexation with metals not only determines metals’ environmental fates (1) but also contributes to stabilize humic structures against biotic and abiotic degradation (2–4). While the role of heavy metals as contaminants had been abundantly investigated (5–7), less work has been devoted to investigate arrangement, solubility, and dynamics of HS when in complexes with divalent and trivalent cations, such as Ca$^{2+}$, Fe$^{3+}$, and Al$^{3+}$ (8–10).

Most abundant divalent and trivalent metals in soils are Ca, Mg, Al, and Fe, thereby influencing humic matter structure to a much greater extent than monovalent cations and other trace elements such as Zn (11). Cations’ chemical nature and HS complexing capacity, due to content and availability of acidic functional groups within humic molecular conformations, determine strength and stability of metal–humic complexes (9,12). Although Ca$^{2+}$ ion appears to bind at different sites of humic structures (9) and has a lower complexing capacity than Al$^{3+}$ ion (1), both metal ions flocculate HS in aqueous media, due to bridging between different negatively charged humic molecules (8,13). Moreover, it has been suggested that hydrolysis of humic matter promotes new high-affinity binding sites, whose saturation by metal ions can prevent further disruption of humic molecular assemblies (14).

A number of methods have been applied to study the changes of humic structures when in complexation with metal ions (15). Advanced X-ray spectroscopy (EXAFS) was found to be sensitive to change in Cu geometry when in transition from aqueous to humic complex state (16). Measurements of time-resolved fluorescence anisotropy revealed alteration of humic conformations in the presence of metal ions (17). A combination of spectroscopic, chromatographic, and capillary electrophoresis—inductively coupled plasma mass spectrometry (CE–ICP-MS) techniques confirmed that humic complexing sites varied with humic samples and their size (18).

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for molecular investigation, but its application to metal–humic complexes has been limited to few studies (19–25). However, literature works report mainly 1D solid-state cross-polarization magic angle spinning (CPMAS) $^{13}$C-NMR or direct spectra of NMR visible metal ions in complexes with humic matter. Measurements of spin–lattice relaxation time in the rotating frame ($T_1\text{(H)}$) of humic suprastructures when in complexes with metals have been obtained from CPMAS-$^{13}$C-NMR spectra for whole soils (24), but the single contribution of humic acids (HA), fulvic acids (FA), and humin (HU) has not been elucidated. Measurement of spin–lattice relaxation time is specifically suitable to evaluate the motion of humic molecules in solid state, and the changes in their rigidity with respect to molecular size and degree of covalent bonds (26,27). Moreover, the $^1$H-DOSY (diffusion order spectroscopy) technique in liquid state is applied to obtain pseudobidimensional NMR spectra that provide information on diffusivity of humic components in solution (28,29). Both these NMR techniques may be applied to follow the effects of metal complexation on HS molecular size, motion, and overall rigidity.

While Fe(III) is paramagnetic and, thus, incompatible with straightforward NMR techniques, other abundant metals, such as Al and Ca, are diamagnetic and compatible with CPMAS- and DOSY-NMR experiments. Al is the most important inorganic component in soil minerals after silicon, and little is known about its interaction with HS (19,20). While Ca does not easily form complexes with organic acids, it shows a good affinity for HS due to their multiple negative surface charges. Ca can significantly affect the HS structure by modifying humic diffusion through the membrane of a two-chamber diffusion cell (30). Moreover, studies by light scattering and differential pulse anodic stripping voltammetry
showed that Ca displaces highly polarizable Cd and Zn from binding with HS (31).

The aim of this work was to investigate the effects of Al and Ca complexation on the structure of a lignite humic acid by means of CPMAS- and DOSY-NMR. Stabilization of humic molecules in metal–humate complexes is related to persistence of organic matter in soils and waters. Further information on metal interactions with humic matter would improve understanding of biogeochemical carbon and nitrogen cycles, as well as emission of C and N greenhouse gases from natural bodies.

Materials and Methods
Humic Matter. A humic acid (HA) was isolated from a North Dakota lignite (Mammoth Chem, Co) and purified as reported elsewhere (32). The HA (1 g) was suspended in 1 L of deionized water and titrated to pH 7 with 0.1 M NaOH for 2 h under a N2 stream, using an automatic titrator (VIT 909 Titrator, Copenhagen). Titration required 2.66 mequiv of NaOH per g of HA, which represented the HA carboxylic acidity (33). In order to eliminate any residual metal cation from the HA, the sodium humate solution was first diluted (1:6) with deionized water and then eluted through a cation exchange resin (Dowex, 50WX2-400) at an approximate resin/HA ratio of 1 g:30 mg. The eluted HA solution was continuously brought back to pH 5 by automatic titration with a 0.1 M NaOH solution and freeze-dried.

Metal Solutions. A 1 g sample of metallic Al (Merck, analytical grade) was suspended in 3.09 mL of 96% H2SO4 (Carlo Erba, reagent grade) and heated to 100 °C to facilitate Al dissolution. The solution was then filtered on a Whatman GF/C quartz filter and diluted to 500 mL. The Al concentration in this Al2(SO4)3 solution was 0.064 M, as determined by AAS (Perkin-Elmer, AA 700). A Ca2+ concentration of 0.01 M was reached by dissolving 147 mg of CaCl2 × 2H2O (Merck, analytical grade) in 100 mL of deionized and MilliQ-filtered water.

Complex Formation. The freeze-dried HA was distributed into 11 aliquots of 90 mg each, which were dissolved in 90 mL of a 0.002 M Na2SO4 (Carlo Erba, reagent grade) solution in order to keep the solution ionic strength constant and low enough not to significantly reduce the Donnan volume and limit changes of HA surface charge distribution (34). Two different series of five of these solutions were added with molecules in metal and Ca complexation on the structure of a lignite humic acid gases from natural bodies. Further information on metal interactions with humic matter would be of great interest for understanding of biogeochemical carbon and nitrogen cycles, as well as emission of C and N greenhouse gases from natural bodies.

Results
Molecular Rigidity. CPMAS-13C NMR spectroscopy is used to characterize the dynamics of solids at the molecular level, being very sensitive to the nanoscale morphology of molecular segments (38). The proton spin–lattice relaxation time in the rotating frame, T1ρ(H), is spatially averaged over several tens of nanometers due to spin diffusion, and depends on molecular morphology. The T1ρ(H) value represents the molar-averaged value for both amorphous and less amorphous, semicrystalline, components of a sample (39). When T1ρ(H) is obtained by variable spin-lock (VSL) NMR experiments, it becomes a measure of the intensity decay for all resolved carbons that are linked to protons, due to processes of polarization transfer and chemical shift. Thus, the T1ρ(H) value for all types of hydrogen allows evaluation of the overall sample mobility. As T1ρ(H) values are also sensitive to proximity of protonated segments to one another, the

Spectra processing was conducted by Mestre-C version 4.9.9.9 software. All free induction decays (FID) were transformed by applying a 2K zero filling and an exponential filter function with a line broadening (LB) of 100 Hz. Manual baseline correction was carried out with a zero-order function using Bernstein polynomials and filter set at the value of 2. For 13C experiments, according to visible signals, spectra were integrated in the intervals of 185–150 ppm (carboxyl carbons), 150–95 ppm (aromatic carbons), and 65–0 ppm (plain or substituted alkyl carbons). The 95–65 ppm (anomeric carbons) region showed no significant signal for this lignite HA. For the 27Al experiment, all visible signals occurred in the same region and around 0 ppm (octahedral Al).
distance between two protonated segments are directly related to proton—proton spin diffusion (40). Consequently, changes in mutual spatial relations of protonated segments in humic complexes with metals may be shown by differences in $T_1(H)$ values as a function of metal content.

The appearance of 1D CPMAS-13C NMR spectra of this lignite HA (32) did not significantly vary with Ca and Al complexation (Figure S1 in the Supporting Information). Moreover, $^{27}$Al-CPMAS-NMR spectra of Al-humates formed at pH 5 distinctly showed a chemical shift for the Al nucleus typical of octahedral coordination (20) in humic complexes, thereby proving the absence of tetrahedral Al oxides (Figure S2 in SI). $T_1(H)$ values, calculated for either the full spectral width or three distinct chemical shift intervals (carboxyl-, aromatic-, and aliphatic-C regions), resulted significantly different with progression of Ca and Al complexation (Table 1).

Relaxation times increased steadily, though not linearly, with content of both Al and Ca for both the full spectral width and chemical shift intervals. The control sample showed the lowest $T_1(H)$ value for the carboxyl-C region, but this value increased sharply for Al—HA complexes at the largest Al saturation (1%). Conversely, Ca did not affect this spectral region to any great extent.

Increase of relaxation time with complexation was significant for the aromatic/double bond-C region (Table 1). The Al complexes showed a significant increase with respect to control. However, while $T_1(H)$ values did not differ from one another at mid-saturation percentages (0.1—0.5%), the largest Al saturation (1%) further increased the $T_1(H)$ value. The HA added with Ca revealed an increase of $T_1(H)$ values over control, which was similar for the low and intermediates Ca saturation (0.05—0.25%), whereas it became significantly large for the most saturated sample. Again, Al complexation gave generally greater $T_1(H)$ values than for Ca addition.

The low-frequency signals in CPMAS-13C NMR spectra (aliphatic-C), most likely long-chained fatty acids as well as small alkyl hydroxylated molecules (41), showed the largest $T_1(H)$ value for control, thereby revealing the most rigid conformation among humic components (Table 1). The Al—HA complexes did not show an increase of the $T_1(H)$ value at low saturation (0.05%), while the values were similarly enhanced over control at larger Al saturations. Addition of Ca to humic samples revealed a significant increase of relaxation times only at the largest Ca saturation (1%). Also for this region, $T_1(H)$ values for Al complexes were invariably greater than for Ca-humates at all metal saturations.

**Molecular Diffusion.** Diffusion-ordered NMR spectroscopy (DOSY) is useful to study aggregation of natural organic matter (28, 29) and interactions of xenobiotics with humic materials (42). DOSY is a pulsed field gradient NMR spectroscopy that enables measurement of translational diffusion of dissolved molecules. The magnitude of diffusion coefficient ($D$) provides direct information on molecular dynamics, including intermolecular interactions, aggregation, and conformational changes (43, 44). Diffusion phenomena are directly related to the hydrodynamic radius of dissolved humic matter in solution and, thus, molecular size (29).

The 2D-DOSY projection of Al- and Ca-humates at different metal content are shown in Figures 1 and 2, respectively. Diffusion coefficients calculated from DOSY spectra for three chemical shift intervals in $^1H$ NMR spectra (aromatic/double bonds−H, 8.0—5.0 ppm; hydroxylalkyl−H, 4.5—2.0 ppm; aliphatic−H, 2.0—0.0) are reported in Table 1. The 2D-DOSY projection of control HA reveals that the 8.0—6.0 ppm region had, on average, larger negative log $D$ values, and, thus, greater association size than the rest of the protons. Addition of 0.05% Al altered such $D$ distribution, suggesting a molecular size decrease of humic molecules with protons in 8.0—7.0 and 4.0—2.0 ppm intervals, whereas components with proton signals between 2.0 and 0.0 ppm increased in molecular size (Figure 1). Addition of 0.1% Al increased values of negative log $D$ for the 7.0—6.0 and 2.0—0.0 ppm intervals, implying an enhanced molecular size of components in these regions, while the aromatic protons in the 8.0—7.0 ppm interval were still diffusing as in smaller-size components. Complexation with 0.25% Al suggested a tendency of molecular reassociation for aromatic components, although aromatic protons resonating at larger frequencies showed a somewhat slower diffusion. At this Al content, the alkyl protons seemed also to diffuse in larger associations than for control and previous Al additions.

Table 1. Values (µs) and Standard Deviation of Spin—Lattice Relaxation Times in the Rotating Frame ($T_{1w}(H)$) for Humic—Al Complexes and Ca-Humates at Varying Percent of Metal Saturation of Humic Carboxyl Acidity As Elaborated from CPMAS-$^{13}$C-NMR Spectra, For Both Total Spectral Width and Range of Chemical Shifts Attributed to Different Carbons

<table>
<thead>
<tr>
<th>metal saturation of COOH sites (%)</th>
<th>carboxyl C (185−150)</th>
<th>aromatic/double bond C (150−95)</th>
<th>aliphatic C (65−0)</th>
<th>total width (185−0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control-0.00</td>
<td>2984 (-149) c</td>
<td>3807 (-152) d</td>
<td>4991 (-100) b</td>
<td>4079 (-163) d</td>
</tr>
<tr>
<td>Al-0.05</td>
<td>3550 (-177) b</td>
<td>4047 (-121) c</td>
<td>5106 (-102) b</td>
<td>4325 (-86) c</td>
</tr>
<tr>
<td>Al-0.10</td>
<td>3475 (-173) b</td>
<td>4459 (-89) b</td>
<td>5650 (-56) a</td>
<td>4691 (-93) b</td>
</tr>
<tr>
<td>Al-0.25</td>
<td>3577 (-178) b</td>
<td>4399 (-175) b</td>
<td>5428 (-217) a</td>
<td>4477 (-179) bc</td>
</tr>
<tr>
<td>Al-0.50</td>
<td>3015 (-150) c</td>
<td>4496 (-89) b</td>
<td>5488 (-55) a</td>
<td>4502 (-30) bc</td>
</tr>
<tr>
<td>Al-1.00</td>
<td>4274 (-213) a</td>
<td>4725 (-94) a</td>
<td>5580 (-111) a</td>
<td>4854 (-145) a</td>
</tr>
<tr>
<td>Ca-0.05</td>
<td>2883 (-144) c</td>
<td>4111 (-123) c</td>
<td>4980 (-100) b</td>
<td>4277 (-128) d</td>
</tr>
<tr>
<td>Ca-0.10</td>
<td>2589 (-129) d</td>
<td>4162 (-124) c</td>
<td>5143 (-154) b</td>
<td>4242 (-127) d</td>
</tr>
<tr>
<td>Ca-0.25</td>
<td>2795 (-139) c</td>
<td>4165 (-83) c</td>
<td>5117 (-102) b</td>
<td>4380 (-131) bc</td>
</tr>
<tr>
<td>Ca-0.50</td>
<td>3064 (-153) c</td>
<td>4467 (-89) b</td>
<td>5078 (-101) b</td>
<td>4447 (-133) bc</td>
</tr>
<tr>
<td>Ca-1.00</td>
<td>3197 (-159) c</td>
<td>4311 (-129) b</td>
<td>5278 (-105) a</td>
<td>4489 (-134) bc</td>
</tr>
</tbody>
</table>

* Different letters in columns indicate significant differences ($P = 0.05$) from control according to the Duncan test.
FIGURE 1. 2D-DOSY projection of control HA and Al-humates at different Al content. Slices A, B, and C refer to $^1$H chemical shift profiles cut through different levels of diffusion axis. Corresponding molecular size decreased in the following order: slice A > slice B > slice C.
FIGURE 2. 2D-DOSY projection of control HA and Ca-humates at different Ca content. Slices A, B, and C refer to $^1$H chemical shift profiles cut through different levels of diffusion axis. Corresponding molecular size decreased in the following order: slice A > slice B > slice C.
of hydrodynamic size. Conversely, diffusion values did not show any significant differences with respect to control for larger metal saturation. As for Ca additions, the $D$ values did not significantly change from that of control at any metal saturation. For the hydroxylalkyl proton region, diffusion coefficients for Al-humic complexes were greater than for control at low (0.05 and 0.1%) and intermediate (0.5%) saturation and suggested a general decrease of molecular size for this class of molecules. The region of nonfunctionalized alkyl protons (2.0–0.0 ppm) showed an opposite trend. The $D$ values significantly decreased, in comparison to control, for both Al- and Ca-humates, thereby indicating that complexation increased the molecular dimension of these alkyl components.

**Discussion**

Our experimental conditions intended to reproduce the environmental interactions of HA with metals. First, an amount of cation greater than 1% of total acidity, the maximum reached here, was rarely found in purified HA isolated from soils (41). Second, mildly acidic pH prevented formation of insoluble Al oxides and concomitantly ensured sufficient deprotonation of carboxyl groups for metal complexation to occur. Third, solution salt content was maintained to minimize changes of ionic strength with metal complexation. Third, solution salt content was maintained to minimize changes of ionic strength with metal complexation. In such conditions, NMR results depended solely on ionic strength variation was less than 10% with respect to control. For both Al- and Ca-humates, thereby indicating that complexation increased the molecular dimension of these alkyl components.

The $T_{1p}(H)$ values indicated that molecular rigidity of classes of humic components was in the following order: aliphatic-C > aromatic/double bonds C > carboxyl-C (Table 1). This is in line with previous findings showing that humic alkyl components are strongly associated in large hydrophobic size aggregates and have great molecular rigidity, while oxidized carbons, being more hydrated, are less tightly aggregated and show less molecular rigidity (27, 29, 45).

However, molecular rigidity of all humic components invariably increased with Al complexation, and slightly less with Ca addition. While interactions of both Al and Ca with humic components are basically electrostatic (34), triple-charged Al ions can form stronger associations with ionized HA groups than divalent Ca ions, by coordinating one more negative charge in a humic molecule than for Ca (10). This may well reflect the different increase of $T_{1p}(H)$ values for Al and Ca humic complexes in both carboxyl- and aromatic/double bonds-C regions. It is likely that saturated and unsaturated long-chain alkanoic acids, which are largely abundant in HS (41), did preferentially form complexes with metals, due to their greater molecular flexibility than stacked aromatic groups (46). The further association of alkanoic acids resulting from mutual complexation of metal cations thus enhanced their conformational stability and molecular rigidity. In fact, internuclear stabilization induced by complexed metals may then favor greater hydrophobic associations among long-chain alkanoic acids and justifies the increase of $T_{1p}(H)$ values with metal addition in the aliphatic-C spectral region (Table 1).

The $T_{1p}(H)$ findings are complementary to those obtained by DOSY-NMR experiments on Al- and Ca-humates (Table 2). These further indicate that not only original humic conformations were altered by metal complexation, but also humic complexes diffusion varied with metal content. At low Al addition, complexation disrupted humic associations into smaller-size aggregates, most probably due to rearrangement of binding energy from weak hydrophobic bonds to strong metal complexes (47). A similar effect was observed by dynamic light scattering when a fulvic acid was made to complex Cd ions (25). However, as Al ions became more available, cross-linkage of binding sites and further complexation enhanced the size of humic aggregates, and their diffusivity was again lowered. This behavior was especially evident for Al-humates in aromatic/double bond spectral intervals, whereas diffusion coefficients for hydroxylalkyl spectral regions remained significantly larger than control also at greater Al addition. This suggests that the most oxidized and hydrated humic components were hardly incorporated in aggregates formed at increasing complexation and showed greater diffusive mobility. Conversely, the strong aggregation induced by metal complexation was particularly effective in spectral regions of hydrophobic aliphatic components, whose decrease in diffusion coefficients generally followed metal content. Ca-humates gave similar DOSY-NMR results, although the disaggregating effect at the smallest Ca addition (0.05%) was not noticed (Table 2), possibly due to the less binding energy of this ion as compared to Al.

The DOSY-NMR results suggest that molecular arrangements of humic molecules were not static, but molecular aggregates of different size were formed at different metal content. In particular, the diffusion behavior of metal–humates revealed that molecules containing aromatic or double bond groups reacted with metals in a different, sometimes opposite, manner than did long-chain alkyl molecules. This implies that humic molecules were not covalently linked to each other, but were rather organized in supramolecular associations.

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**Table 2. Diffusion Coefficients (10^-10 m^2/s) for Humic–Al Complexes and Ca-Humates at Varying Percent of Metal Saturation of Humic Carboxylic Acidity As Elaborated from DOSY Spectra for Range of Chemical Shifts Attributed to Different Protons**

<table>
<thead>
<tr>
<th>metal saturation of COOH sites (%)</th>
<th>diffusion coefficients (10^-10 m^2/s)</th>
<th>aromatic/double bond H (8–5.0)</th>
<th>hydroxylalkyl H (4.5–2.0)</th>
<th>aliphatic H (2.0–0.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control-0.00</td>
<td>1.16 ± 0.04 b</td>
<td>1.42 ± 0.05 b</td>
<td>1.54 ± 0.08 a</td>
<td></td>
</tr>
<tr>
<td>Al-0.05</td>
<td>1.45 ± 0.07 a</td>
<td>1.68 ± 0.13 a</td>
<td>1.32 ± 0.08 b</td>
<td></td>
</tr>
<tr>
<td>Al-0.10</td>
<td>1.36 ± 0.10 a</td>
<td>1.66 ± 0.07 a</td>
<td>1.25 ± 0.06 b</td>
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</tr>
<tr>
<td>Al-0.25</td>
<td>1.18 ± 0.12 b</td>
<td>1.43 ± 0.10 b</td>
<td>1.37 ± 0.05 b</td>
<td></td>
</tr>
<tr>
<td>Al-0.50</td>
<td>1.24 ± 0.09 b</td>
<td>1.66 ± 0.11 a</td>
<td>1.32 ± 0.02 b</td>
<td></td>
</tr>
<tr>
<td>Al-1.00</td>
<td>1.21 ± 0.03 b</td>
<td>1.50 ± 0.06 b</td>
<td>1.26 ± 0.14 b</td>
<td></td>
</tr>
<tr>
<td>Ca-0.05</td>
<td>1.24 ± 0.01 b</td>
<td>1.54 ± 0.06 b</td>
<td>1.24 ± 0.04 b</td>
<td></td>
</tr>
<tr>
<td>Ca-0.10</td>
<td>1.20 ± 0.07 b</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>Ca-0.50</td>
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<td>1.04 ± 0.13 c</td>
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</tr>
<tr>
<td>Ca-1.00</td>
<td>1.22 ± 0.18 b</td>
<td>1.48 ± 0.17 b</td>
<td>1.26 ± 0.20 b</td>
<td></td>
</tr>
</tbody>
</table>

* Standard deviation was from 0.01 to 0.2 x 10^-10 m^2/s for all samples. Different letters in columns indicate significant differences ($P = 0.05$) from control according to the Duncan test.
and specifically responded to chemical changes brought about by metal additions.

The NMR techniques applied here enlarged the understanding of molecular changes occurring in humic superstructures when in metal complexes. This NMR approach proved to be sensitive to changes in rigidity and hydrodynamic volume of humic metal complexes within different spectral regions. Our results showed that it is possible to reach a molecular characterization of the humic sites responsible for metal complexation. The humic carboxyl groups were confirmed to be responsible for Al and Ca complexation. However, being part of specific molecules within humic superstructures, their complexation and metal determined the rearrangement of functional molecular interactions due to new conformational stability. Aromatic or double bond groups showed a different response to metal complexation than did alkyl components, the latter becoming generally more strongly associated than the former. These NMR results are useful to further explain the molecular bases of complex formation between humic molecules and metals and contribute to understanding the selective environmental persistence of specific classes of carbonaceous compounds.

Acknowledgments
The first author is a Ph.D. candidate of the Doctorate School “Valorizzazione e Gestione delle Risorse Agro-forestali” of the Università di Napoli Federico II. Useful comments from Dr. D. Šmejkalová are gratefully appreciated.

Supporting Information Available
Investigation on 13C-CPMAS-NMR spectroscopy of Al– and Ca–HA complexes (1% of metal saturation) and on 27Al-CPMAS-NMR spectroscopy of HA–Al complex (1% of metal saturation). This material is available free of charge via the Internet at http://pubs.acs.org.

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ES802807Y