Relating Ion Binding by Fulvic and Humic Acids to Chemical Composition and Molecular Size. 2. Metal Binding

4 AUTHORS, INCLUDING:

Ruben Kretzschmar
ETH Zurich
234 PUBLICATIONS **7,045** CITATIONS

Relating Ion Binding by Fulvic and Humic Acids to Chemical Composition and Molecular Size. 2. Metal Binding

ISO CHRISTL,[†] CHRIS J. MILNE,[‡]
DAVID G. KINNIBURGH,[‡] AND
RUBEN KRETZSCHMAR*,[†]

Institute of Terrestrial Ecology, Swiss Federal Institute of Technology, Grabenstrasse 3, CH-8952 Schlieren, Switzerland, and British Geological Survey, Wallingford, Oxon, OX10 8BB, U.K.

Binding of Cu(II) and Pb(II) to a soil fulvic acid, humic acid, and two different size fractions of the humic acid was investigated with metal titration experiments at pH 4, 6, and 8. Proton and free metal ion activities in solution were monitored after each titration step using pH and ion selective electrodes (ISE), respectively. The amounts of base required to maintain constant pH conditions were recorded and used to calculate stoichiometric proton-to-metal ion exchange ratios. Despite clear differences in chemical composition and protonation behavior, the fulvic acid and all humic acid fractions exhibited very similar metal binding behavior. Binding of Cu(II) and Pb(II) generally increased with increasing pH and total metal concentration. At low to moderate metal ion concentrations, Cu(II) was bound more strongly to the humic substances than Pb(II). Only at high free metal concentrations, the amounts of metal ions sorbed were higher for Pb(II) than for Cu(II). The molar proton-to-metal ion exchange ratios ranged from 1.0 to 1.8 for Cu(II) and from 0.6 to 1.2 for Pb(II), suggesting that Cu(II) was bound as monodentate and bidentate complexes, while Pb(II) was bound predominantly as monodentate complexes. The metal ion binding data were quantitatively described with the consistent NICA-Donnan model. The best description of an entire multicomponent data set consisting of proton titration, Cu(II), and Pb(II) binding data was achieved when the entire data set was fitted simultaneously. To reduce the number of fitting parameters, results from size exclusion chromatography and solid state ^{13}C NMR spectroscopy were used to estimate two of the NICA-Donnan model parameters. The values of the remaining NICA-Donnan parameters for the humic substances are within a narrow range, suggesting that generalized model parameters may be useful in geochemical modeling involving humic substances.

Introduction

Humic and fulvic acids are polydisperse mixtures of natural organic polyelectrolytes containing a large number of dif-

ferent functional groups (*1*). Studies on the chemical characterization of humic and fulvic acids indicate that the amounts of different functional groups vary significantly between humic substances of different origin. However, only few studies have been conducted on chemical differences between molecular size fractions of humic substances (*2*). In Part 1 of this series (*3*), we demonstrated that differences in proton binding behavior of the fulvic acid and the humic acid size fractions isolated from a surface soil are consistent with chemical characterization results. The NICA-Donnan model excellently described proton binding data of humic acid size fractions when some model parameters were related to characterization results. Since cation binding to humic and fulvic is recognized to be mainly due to the presence of acidic functional groups such as, e.g., carboxylic and phenolic groups (*4*), it has been hypothesized that the size of humic molecules would affect trace metal complexation (*5*). However, detailed investigations on metal ion binding to humic substances in relation to chemical composition are lacking. A better understanding of metal ion binding to humic substances is of fundamental importance in geochemical modeling of near-surface environments, where natural organic matter is always present and represents an important sorbent for metal ions. Development of a generalized database of model parameters for metal ion binding to humic substances has been hampered by the assumption that humic substances are too heterogeneous in nature. However, if the influence of chemical composition on metal binding is only weak, as proposed by Avena et al. (*6*), average stability constants for different metal humate complexes derived from previous studies may be useful for predicting the fate of metal cations at contaminated sites. On the other hand, if differences in chemical composition result in large differences in metal ion binding, a generalized database would not be useful in most cases.

The objectives of this paper are (1) to study the pH-dependent binding of Cu(II) and Pb(II) to a set of well characterized soil fulvic acid and humic acid size fractions differing in their chemical composition, (2) to model the metal ion binding data using the NICA-Donnan approach, and (3) to relate model parameters to chemical characterization results for the same humic substances reported in previous papers (*3, 7*).

Experimental Section

Preparation of Humic Substances. Humic and fulvic acids were extracted from a well humified organic horizon (H) of a Humic Gleysol near Unterrickenzopfen (northern Switzerland) and purified following a standard procedure of the International Humic Substances Society (*7*). The humic acid fraction was further fractionated into four molecular size classes with nominal molecular weights of 10–30 kD, 30–100 kD, 100–300 kD, and >300 kD using a hollow fiber ultrafiltration technique (*7*). A detailed description of the extraction, purification and ultrafiltration procedures is given elsewhere (*7*). All fractions were characterized by a combination of methods including FT-IR, CP-MAS ^{13}C NMR, and elemental analysis (*7*). Characterization results are summarized and discussed in relation to proton binding in Part 1 of this series (*3*). It was shown that the number of functional groups capable of binding protons increased with decreasing average molecular weight of the humic acid size fractions. Accordingly, the negative charge of the humic acid fractions increased with decreasing average molecular weight. The results further suggested that the amounts of carboxylic and

* Corresponding author phone: +41-1-6336003; fax: +41-1-6331118; e-mail: kretzschmar@ito.umnw.ethz.ch.
† Institute of Terrestrial Ecology.
‡ British Geological Survey.

phenolic functional groups determined the charging behavior of the fulvic and humic acid fractions.

For the investigation of Cu and Pb binding, we confined ourselves to the fulvic acid (FA), the total humic acid (HA), and the humic acid size fractions 30–100 kD ($HA_{30-100 \ kD}$) and >300 kD ($HA_{>300 \ kD}$). The humic acid fractions 10–30 kD and 100–300 kD were omitted as these fractions contributed less than 10% of the total mass of soil humic acid.

Calibration of Ion-Selective Electrodes. Ion-selective electrodes (ISE) for Cu^{2+} (Orion 9429) and Pb^{2+} (Orion 9482) were calibrated by titration of metal salt solutions with an organic ligand. It has been shown that the calibration range commonly reported for sulfide-based solid-state ISEs is extendable to lower metal activities by orders of magnitude by means of titration with a strong ligand compared to simple calibrations using unbuffered solutions of different metal salt concentrations (8–12). Both Cu^{2+} and Pb^{2+} sensitive electrodes were calibrated by titrating 50 mL of a 0.15 mM $Cu(NO_3)_2$ (Merck, p.a.) and $Pb(NO_3)_2$ (Merck, p.a.) solution, respectively, with a 1.787 mM ethylenediamine (p.a., Fluka) solution using a computer controlled titration system (13). The calibration was conducted in a thermostated room at 25 ± 1 °C. The ethylenediamine solution was prepared under nitrogen gas atmosphere using CO_2-free deionized water. To exclude CO_2 during the calibration, the titration vessel was sealed and flushed with water-saturated, CO_2-free nitrogen gas. To maintain a constant ionic strength throughout the calibration, metal salt and ethylenediamine solutions were prepared in a 0.1 M $NaNO_3$ (Merck, p.a.) background. Prior to recording electrode readings, the metal solution was stirred for 2 min after each titrant addition. Readings of a pH electrode (6.0123.100, Metrohm) and the respective ISE were measured against an Ag/AgCl reference electrode (6.0733.100, Metrohm) placed into an electrolyte bridge containing a 0.1 M $NaNO_3$ solution which was continuously replaced by a peristaltic pump. Electrode readings were recorded when both potential drifts had dropped below 0.1 mV min^{-1} or after a maximum equilibration time of 30 min. Solution speciation was calculated for each recorded data point using the chemical speciation program ECOSAT (14). Hydrolysis constants of aqueous metal species were taken from Baes and Mesmer (15). Critical stability constants of ethylenediamine complexes as well as solubility constants of metal oxide and hydroxide phases were taken from Smith and Martell (16, 17). The calculated free metal ion activities were linearly related to the measured ISE voltage readings down to pCu 17 and pPb 9.5, where pMe is the negative logarithm of the free metal ion (Me^{2+}) activity in solution. The performance of both ISEs used was checked before and after each fulvic and humic acid titration. Fulvic and humic acid titration data were used for further analysis only if the calibration before and after each experiment did not differ significantly and if the response of the electrodes was nearly Nernstian.

Copper and Lead Binding to Fulvic and Humic Acid. The binding of Cu(II) and Pb(II) to the fulvic acid and the humic acid size fractions was measured at constant ionic strength (0.1 M $NaNO_3$) and constant pH values (pH 4, 6, and 8). All experiments were performed in a thermostated room at 25 ± 1 °C using the same titration setup as for ISE calibration (13). The four burets were filled with 0.05 M HNO_3 (Titrisol, Merck), ~0.05 M NaOH, 1 mM $Cu(NO_3)_2$ (Merck, p.a.) containing 0.1 M $NaNO_3$ (Merck, p.a.), and 0.1 M $Cu(NO_3)_2$ (Merck, p.a.) for Cu titrations. For Pb binding experiments, $Pb(NO_3)_2$ (Merck, p.a.) solutions were used instead of $Cu(NO_3)_2$ solutions. NaOH and metal solutions were prepared under nitrogen gas atmosphere using CO_2-free deionized water. To keep the solutions CO_2-free, the burets were connected with the atmosphere only through a glass tube filled with NaOH on granulated activated carbon

(Merck, p.a.). All experiments were carried out in a 200 mL glass vessel, which was continuously flushed with water-saturated, CO_2-free nitrogen gas. Each experiment was started with a 100 mL solution containing 0.1 M $NaNO_3$ and approximately 100 mg of humic or fulvic acid. To ensure complete dispersion of the humic acid, the pH was first increased to pH 10 by NaOH addition and then lowered to the desired value by HNO_3 addition. Before starting metal titrations, the pH was kept constant within a tolerance range of ±0.004 pH units (0.2 mV) for 12 h. This procedure was used to fully equilibrate the humic acid to pH and ionic strength conditions before metal addition was started. During metal titrations, the humic solutions were stirred for 4 min after each addition of titrant. After metal addition, the pH was automatically readjusted to the desired pH value and then kept constant for 20 min within a tolerance range of ±0.004 pH units (0.2 mV). Electrode readings were recorded when the potential drift was below 0.05 mV min^{-1} for the pH electrode and 0.1 mV min^{-1} for the ISE or after a maximum equilibration time of 20 min. On average, readjustment of the pH value after metal addition took 60–75 min. Doses of metal salt additions were gradually increased during the experiments. To cover a large free metal ion concentration range in one titration experiment without exceeding the maximum volume of the titration vessel, 20 mL of 1 mM metal solution was added stepwise. Then, a 0.1 M metal solution was used for further metal addition. Each experiment was automatically stopped at a critical mV reading of the ISE, which corresponded to a free metal activity close to the level where metal (hydr-)oxide precipitation must be expected according to chemical speciation calculations. During each experiment, the ionic strength remained constant within 3%.

Data Analysis. For each titration data point, the amount of inorganic species in solution was calculated using the chemical speciation program ECOSAT (14). Metal hydrolysis constants and metal (hydr-)oxide solubility constants given by Baes and Mesmer (15) and Smith and Martell (17) were used in the calculations. For the calculation, pH, metal activity, and concentration of sodium and nitrate were used as input data. The amount of metal sorbed to the respective humic substance was calculated by subtracting the sum of aqueous metal species from the known total amount of metal in solution. Stoichiometric exchange ratios for proton and metal ions were calculated from the increase of metal sorption and the amount of base needed to readjust pH after metal addition. The calculation was corrected for hydroxide consumption of the bulk solution due to pH adjustment and formation of hydrolysis species of Cu and Pb.

Data Modeling

Experimental Cu(II) and Pb(II) binding data were fitted using the consistent nonideal competitive adsorption (NICA) model (18). Electrostatic interactions were accounted for using the Donnan gel approach (1). The NICA-Donnan model was chosen for this study, because it has been quite successful in describing proton and metal binding to different humic substances, taking the chemical heterogeneity and the gel structure of humic substances into consideration (3, 18–20). A brief summary of the NICA-Donnan model has been given in Part 1 of this series (3), and a complete description can be found in Kinniburgh et al. (18).

Copper and lead sorption data were simultaneously fitted with proton binding data presented in Part 1 of this series (3). The NICA-Donnan model contains a large number of adjustable parameters. One of the objectives of this study was to reduce the number of fitting parameters by relating chemical characterization results to proton and metal binding data modeled with the NICA-Donnan model. Therefore, the amount of high affinity binding sites, $Q_{max,2}$ was fixed to the amount of phenolic carbon determined by solid-state ^{13}C

NMR spectroscopy (*3*). In addition, the Donnan parameter *b* was estimated from size exclusion chromatograms (*3*).

The stoichiometry of proton-metal ion exchange provides additional information about the dentism of divalent metal cation binding to humic substances. The proton-metal ion exchange ratio, r_{ex}, is given by

$$r_{ex} = -\frac{(\partial H_b/\partial H)}{(\partial M_b/\partial H)} \quad (1)$$

where *H* is the proton concentration in solution, and H_b and M_b are the amount of protons and metal ions bound to the humic substances, respectively. In a system containing only two specifically sorbing ions, e.g., protons and one type of metal ion, r_{ex} can be calculated from the NICA-Donnan model parameters (*18*)

$$r_{ex} =$$

$$\frac{n_H[(\tilde{K}_H H)^{n_H}\{1 + [(\tilde{K}_H H)^{n_H} + (\tilde{K}_M M)^{n_M}]\} - p(\tilde{K}_H H)^{n_H}]}{n_M[(\tilde{K}_H H)^{n_H}\{1 + [(\tilde{K}_H H)^{n_H} + (\tilde{K}_M M)^{n_M}]\} + p(\tilde{K}_M M)^{n_M}]}$$
$$(2)$$

where *M* is the metal ion concentration in solution, n_i is the ion-specific heterogeneity parameter of species *i*, *p* is a parameter reflecting the intrinsic heterogeneity of the humic substances, and $\tilde{K}_i$ is the median value of the affinity distribution constants for ion i.

Results and Discussion

Copper Binding. Binding isotherms of Cu to fulvic acid, humic acid, and two different humic acid size fractions ($HA_{30-100\,kD}$ and $HA_{>300\,kD}$) are presented in Figure 1. All binding isotherms plotted on a log−log scale were nearly linear at free Cu ion concentrations below 10^{-6} M. At high concentrations, the isotherms tended to exhibit saturation with a maximum adsorption capacity of roughly 3 mol kg^{-1}. Due to proton-metal ion competition for binding sites, metal ion sorption decreased with decreasing pH. In addition, the increase of charge of humic and fulvic acids with increasing pH (*3*) may cause an increase of metal sorption.

A comparison of Cu binding to humic acid size fractions (Figure 1) reveals that the amounts of Cu bound by the two size fractions $HA_{30-100\,kD}$ and $HA_{>300\,kD}$ are nearly identical, although $HA_{30-100\,kD}$ contains more functional groups and is more negatively charged than $HA_{>300\,kD}$ (*3*). Only at high Cu concentration (nonlinear part of isotherms of log−log plots), a significantly greater amount of Cu is bound by the lower molecular weight fraction. This demonstrates that the effect of differences in chemical composition of humic acid size fractions is small as long as enough sites are available to bind Cu ions. At high Cu concentrations, differences between the two humic acid size fractions can be observed as the smaller fraction exhibits a higher maximum binding capacity due to a greater number of functional groups.

The amounts of Cu bound to fulvic and humic acid are fairly similar at pH 4. At pH 6 and 8, Cu is bound more strongly by humic acid than by fulvic acid. At high Cu concentrations, the differences between fulvic and humic acid isotherms at pH 6 and 8 become smaller. This result indicates that it is not only the total amount of different functional groups that affects the Cu binding behavior of humic substances. Chemical structure, and in particular the linkage of the functional groups to the carbon backbone, may have a strong influence, too. Fluorescence spectra of humic and fulvic acid suggested that a greater percentage of carboxyl groups in fulvic acid are linked to aliphatic backbones than to aromatic ring systems compared to humic acid (*7*). This means that the probability of adjacent carboxyl and phenolic groups on
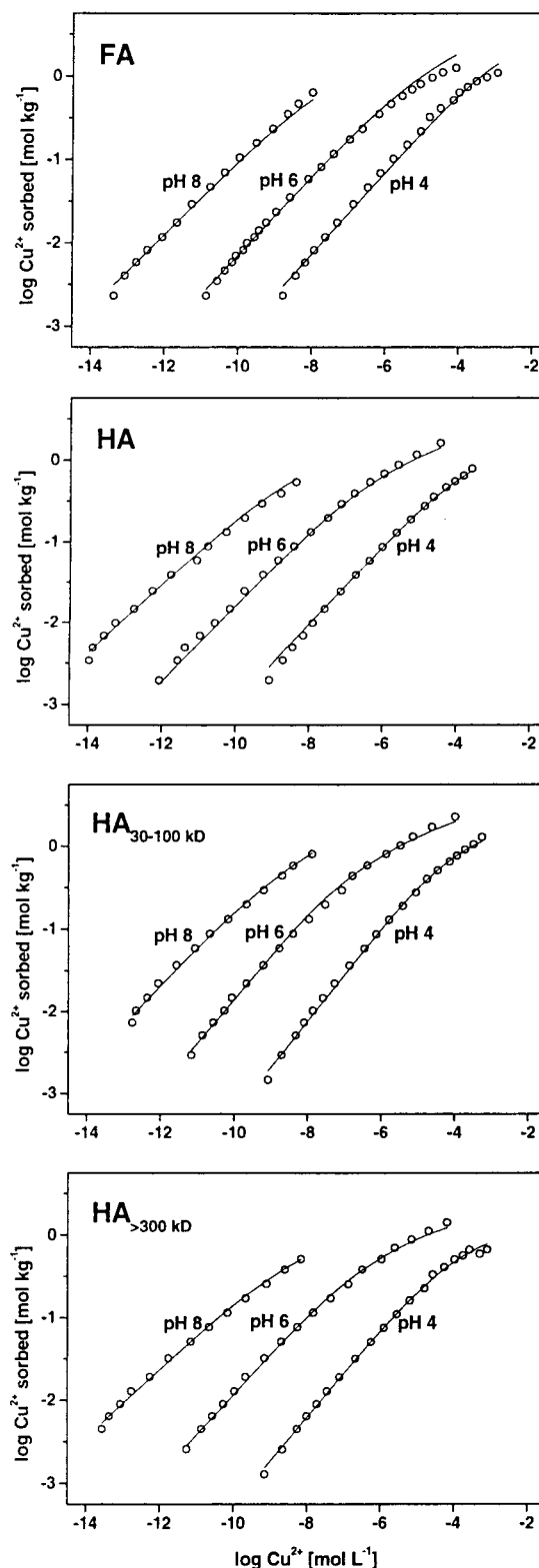

FIGURE 1. Copper binding to fulvic acid (FA), humic acid (HA), and humic acid size fractions at pH 4, 6, and 8 in 0.1 M NaNO₃ plotted against the free metal ion concentration. Symbols represent data calculated from metal titrations. Lines represent fits obtained with the consistent NICA-Donnan model (see Table 1 for parameter values).

aromatic rings, such as in phthalic or salicylic acid, capable of forming strong complexes with Cu^{2+} is higher for humic acid than for fulvic acid. The fact that the isotherms of humic and fulvic acid tend to merge at high Cu concentrations again
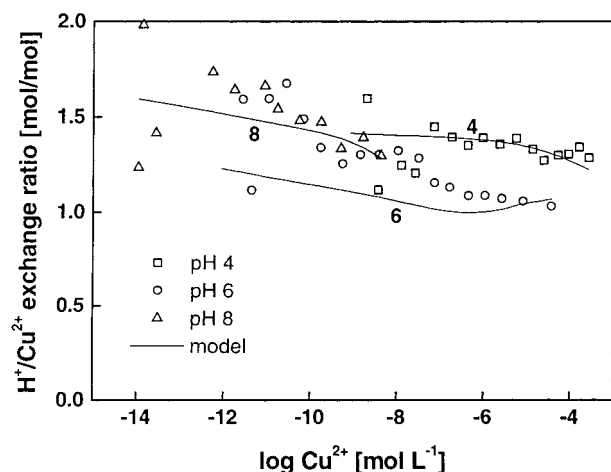
FIGURE 2. Molar H^+/Cu^{2+} exchange ratio for Cu binding to humic acid at pH 4, 6, and 8 in 0.1 M $NaNO_3$ plotted against the free metal ion concentration. Symbols represent data calculated from metal titrations. Lines represent predictions obtained with the consistent NICA-Donnan model.

may be due to the greater amount of binding sites of fulvic acid compensating for the influence of the structural differences. Overall, Cu binding data of fulvic acid, humic acid, and humic acid size fractions indicate that amounts, types, and linkage of functional groups affect Cu binding behavior. At high Cu concentrations, the total amount of binding sites may be the most important factor.

Proton-to-copper ion exchange ratios of fulvic acid, humic acid, and humic acid size fractions derived from Cu titration data range from 1.0 to 1.8. The values vary with pH and Cu concentration. As an example, H^+/Cu^{2+} exchange ratios of humic acid are shown in Figure 2. The range of the values implies that protons are exchanged by Cu in a 1:1 and in a 2:1 stoichiometry. This suggests that Cu is bound by humic substances as monodentate and bidentate complexes. Similar proton-copper ion exchange ratios have been found for a purified peat (*12*) and a soil humic acid (*21*).

Lead Binding. Binding isotherms for Pb to fulvic acid, humic acid, and humic acid are shown in Figure 3. Again, a decrease in Pb binding with decreasing pH is observed, due to competition of Pb ions with protons for binding sites. The binding of Pb to humic acid size fractions was almost identical. Even at high Pb concentrations, only small differences in Pb binding behavior of humic acid size fractions were observed. In contrast to Cu binding, significant differences in Pb binding behavior of humic and fulvic acid were observed at pH 4 but not at pH 6 and 8. A comparison of Pb binding to fulvic and humic acid with other humic and fulvic acids reported in the literature reveals that the results of this study fit very well to results of previous studies (*20, 22, 23*). This suggests that Pb binding to humic substances is nearly unaffected by differences in chemical composition of humic substances.

Compared to Cu binding (Figure 1), Pb binding is affected more strongly by variations in the metal ion concentration (Figure 3). This means that the slope of the sorption isotherms is greater for Pb than for Cu. Pb sorption isotherms of all humic substances studied intersect the respective Cu isotherms at high metal ion concentrations. Thus, more Pb than Cu is bound by humic substances at very high respective metal ion concentrations, whereas the amount of Cu sorbed exceeds the amount of Pb sorbed by orders of magnitudes at low metal ion concentrations, which are environmentally more relevant.

Proton-to-lead ion exchange ratios of fulvic acid, humic acid, and humic acid size fractions are fairly similar. The
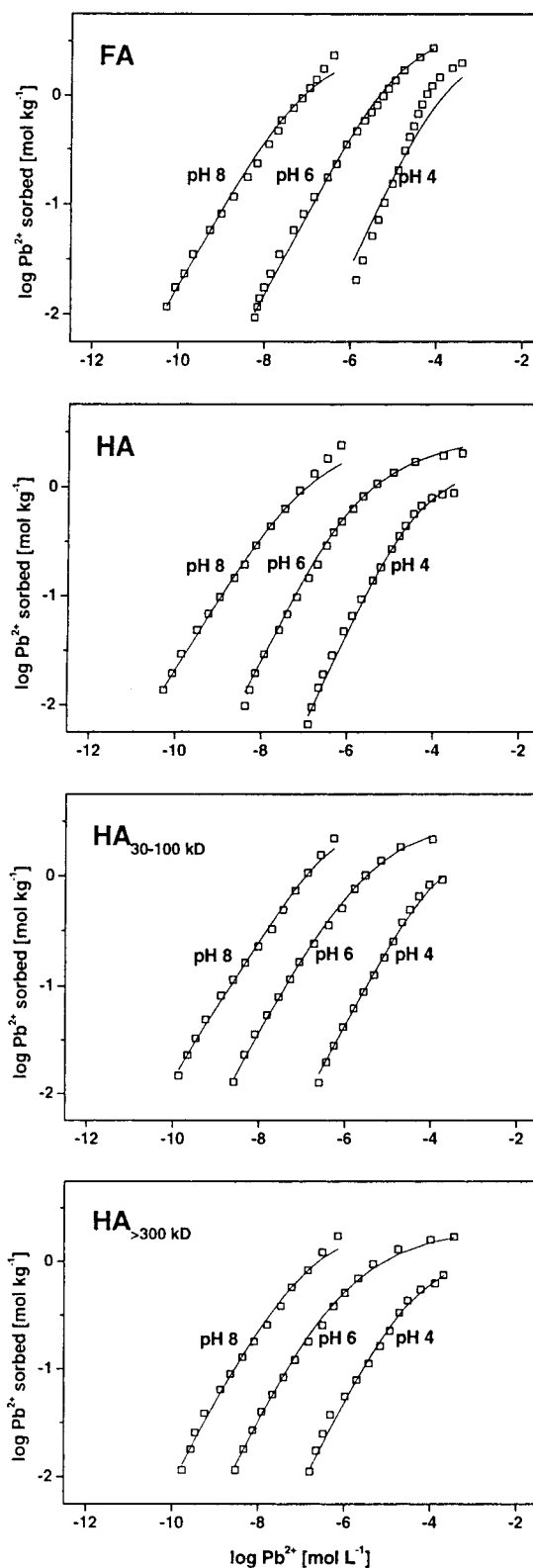

FIGURE 3. Lead binding to fulvic acid (FA), humic acid (HA), and humic acid size fractions at pH 4, 6, and 8 in 0.1 M $NaNO_3$ plotted against the free metal ion concentration. Symbols represent data calculated from metal titrations. Lines represent fits obtained with the consistent NICA-Donnan model (see Table 1 for parameter values).

values range from 0.6 to 1.2. As an example, H^+/Pb^{2+} exchange ratios of the total humic acid fraction are shown in Figure 4. As for Cu, H^+/Pb^{2+} exchange ratios vary with pH and metal ion concentration. The values of the exchange ratios agree
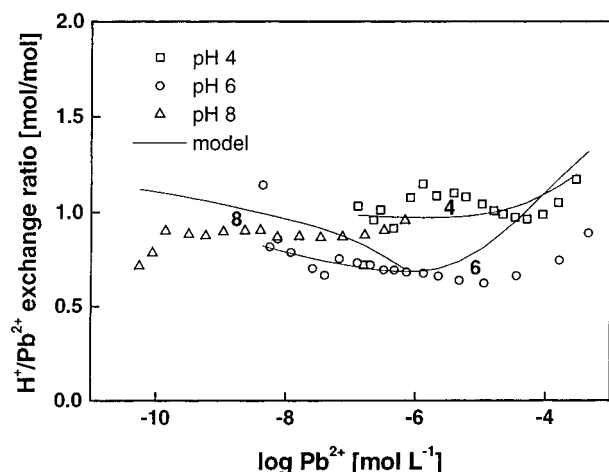
FIGURE 4. Molar H^+/Pb^{2+} exchange ratio for Pb binding to humic acid at pH 4, 6, and 8 in 0.1 M $NaNO_3$ plotted against the free metal ion concentration. Symbols represent data calculated from metal titrations. Lines represent predictions obtained with the consistent NICA-Donnan model.

TABLE 1. NICA-Donnan Model Parameters of Soil Organic Acid Fractions Studied[a]

	fulvic acid	humic acid	humic acid >300 kD	humic acid 30−100 kD
b^b	*0.57*	*0.40*	*0.30*	*0.51*
Q_{max1} [mol kg^{-1}]	6.0	3.1	2.1	3.2
p_1	0.67	0.61	0.62	0.53
log $\tilde{K}_{H1}$	2.4	2.8	2.9	2.8
n_{H1}	0.65	0.79	0.84	0.93
log $\tilde{K}_{Cu1}$	0.0	1.5	1.8	2.0
n_{Cu1}	0.50	0.50	0.55	0.58
log $\tilde{K}_{Pb1}$	0.6	1.7	1.8	1.6
n_{Pb1}	0.90	0.90	0.79	0.74
Q_{max2} ^{c} [mol kg^{-1}]	*2.7*	*2.7*	*2.2*	*4.0*
p_2	0.30	0.30	0.30	0.23
log $\tilde{K}_{H2}$	8.7	8.0	7.3	9.0
n_{H2}	0.80	0.80	0.78	0.74
log $\tilde{K}_{Cu2}$	8.6	8.1	6.8	8.5
n_{Cu2}	0.44	0.41	0.40	0.44
log $\tilde{K}_{Pb2}$	6.6	5.8	4.2	5.4
n_{Pb2}	0.70	0.63	0.86	0.93
R^2	0.9983	0.9978	0.9986	0.9980

^{a} Results were obtained from simultaneous fits of proton, copper, and lead binding data. Fixed NICA-Donnan model parameters are in italics. ^{b} Donnan volume parameters b were fixed to values related to molecular weight estimations (*3*). ^{c} Q_{max2} was fixed to the amount of phenolic carbon derived from ^{13}C NMR spectra and carbon contents determined by elemental analysis (*7*).

well to H^+/Pb^{2+} exchange calculated for a peat humic acid (*18*). The range of H^+/Pb^{2+} exchange ratios suggests that Pb binding reactions follow a 1:1 stoichiometry, predominantly. Differences in the stoichiometry of binding reactions imply that bidentate binding sites are less important for Pb binding compared to Cu binding. For Pb, the total number of binding sites may be the main factor controlling the binding behavior. The structural configuration of binding sites may be secondary for Pb binding. This may be the reason Pb binding of humic acid size fractions is nearly unaffected by chemical differences between humic acid size fractions, while Cu binding is affected. The fact that proton-metal ion exchange ratios of humic substances are lower for Pb than for Cu may further explain differences in the aggregation of humic substances with Pb and Cu, respectively. Saar and Weber (*24*) found that Pb-fulvate precipitated at much lower mole ratio of metal ion to fulvic acid than Cu-fulvate. We observed the same phenomenon during our experiments. The stability of fulvic colloids is, like the stability of any other colloid, strongly influenced by the charge of the colloids (*25*). Since one proton is exchanged by one Pb^{2+} ion, on average, whereas one Cu^{2+} ion exchanges about 1.5 protons, the negative charge of fulvate is compensated by Pb binding more than by Cu binding. The dependence of the aggregation behavior on the metal cation implies that the mobility of metal-organic compounds in natural systems may be quite different for different metal cations (*26*).

NICA-Donnan Modeling. The NICA-Donnan model provided excellent descriptions of Cu (Figure 1) and Pb binding (Figure 3) to fulvic acid, humic acid, and humic acid size fractions ($R^2 \geq 0.9978$). Values of R^2 based on simultaneous fits of proton, Cu, and Pb binding data are shown in Table 1. Only, the slope of the pH 4 Pb isotherm of fulvic acid differing significantly from the slope of pH 6 and 8 isotherms was not satisfactorily described (Figure 3). In addition, high Pb loadings of fulvic acid, humic acid, and humic acid size fractions at pH 8 were not fitted accurately. This mismatch of the model cannot be solved by weighting data points, as the model performance at high metal loadings is strongly limited by the maximum sorption capacity which is the same for each pH value. However, one has also to keep in mind that error of high metal loading data points may be greater than for low metal loadings (*27*).

To relate the model parameters to chemical characterization results of the fractions investigated and to reduce the number of adjustable parameters, Q_{max2} was fixed to the

amount of phenolic carbon calculated from ^{13}C NMR spectra and carbon contents. Furthermore, the Donnan volume parameter b was estimated from peak elution times measured by size exclusion chromatography (*3*). Adjusting Q_{max2} and b did not result in a substantial improvement of the model fits.

All model descriptions presented in Figures 1 and 3 are the results of simultaneous fits of proton binding data given in the anterior paper (*3*) and Cu and Pb binding data. For all humic substances studied, only the simultaneous fits of the entire multidimensional data sets provided excellent description of proton and metal ion binding. Successive data modeling, that is deriving protonation constants and number of binding sites Q_{max1} and Q_{max2} from acid−base titration data, keeping these values fixed, and then fitting metal binding data, i.e., first Cu and finally Pb or vice versa, led to unsatisfactory descriptions of the entire data set. Successive data fitting resulted in R^2 values for metal binding of less than 0.85, whereas the simultaneous fitting procedure yielded R^2 values above 0.98 (Table 1). From our results we conclude that the number of binding sites, Q_{max1} and Q_{max2} as well as the median protonation constants of the NICA-Donnan model cannot be reliably derived for humic substances from acid−base titration alone. Simultaneous fitting of a data sets and deriving model parameters from characterization results as far as possible seems to be the most promising way to obtain good modeling results for the entire data set with physically and chemically reasonable model parameter values. Additional constraining such as relating model parameters to characterization results makes the fitting procedure more stable and the results more reliable (*3*).

Based on fits of metal ion binding data sets, the proton-metal ion exchange ratios were predicted for Cu and Pb (eq 2). Proton-metal ion exchange ratios calculated from metal titration data and predicted with the NICA-Donnan model are shown for the total humic acid fraction (Figures 2 and 4) as an example for all fractions investigated. The predicted proton-metal ion exchange ratios were in good agreement with data calculated from experimental data. This confirms that the NICA-Donnan model can comprehensively describe proton and metal ion binding by humic substances.

The complete NICA-Donnan model parameter set for proton, Cu, and Pb binding to fulvic acid, humic acid, and humic acid size fractions are given in Table 1. The values of proton binding constants have been discussed in the anterior paper (3). The values of $\tilde{K}_{Cu1}$ and $\tilde{K}_{Pb1}$, the median Cu and Pb affinity constants of low proton affinity sites (carboxylic type), are in the same order of magnitude for humic acid and humic acid size fractions. For fulvic acid, both values are smaller by about 1 order of magnitude. This seems reasonable because of the differences in linkage of carboxylic groups of fulvic and humic acid (7). Since carboxylic groups of fulvic acid are more likely to be linked to aliphatic carbon backbones, while more carboxylic groups are linked to aromatic systems for humic acid, humic acid may contain more salicylate and phthalate like parts which are generally recognized to be predominant metal binding sites of humic substances ($28, 29$). In general, the affinity of carboxylic type binding sites for Cu and Pb are similar for the humic substances investigated. Pb binding constants tend to be slightly higher than Cu binding constants of carboxylic sites for fulvic and humic acid.

For high proton affinity sites (phenolic type), median metal ion binding constants are much greater for Cu than for Pb (Table 1). On average, Cu binding constants exceed Pb binding constants by 2 orders of magnitudes. In contrast to low proton affinity sites, Cu and Pb binding constants of high proton affinity sites are greater for fulvic acid than for humic acid. In addition, clear differences in metal ion binding constants of high proton affinity sites can be seen for humic acid size fractions. Values of $\tilde{K}_{Cu2}$ and $\tilde{K}_{Pb2}$ are much higher for $HA_{30-100 \text{ kD}}$ compared to $HA_{>300 \text{ kD}}$.

A comparison of NICA-Donnan model parameters of fulvic and humic acid with parameters reported in the literature ($18, 20, 30$) shows that not only binding constant but also heterogeneity parameters p_i and n_i fitted are in general agreement with values reported previously for fulvic and humic acids, respectively. However, differences between the parameter sets derived for different humic substances are significant. Thus, investigation of proton and metal ion binding behavior seems to be essential to obtain model parameters able to accurately describe and predict metal ion sorption to distinct humic substances. Incorporation of characterization results appears be a good means for deriving physically and chemically reliable parameters. However, detailed characterization of humic substances and precise investigation of proton and metal ion binding is very time-consuming. Since NICA-Donnan model parameters derived for different humic substances do not differ widely, generalized (average) NICA-Donnan parameter may be used to estimate metal binding of humic substances. As organic matter is a major sorbent of metal cations in natural systems, it has to be included in geochemical modeling. For the application of geochemical models to environmental systems, a database of generalized NICA-Donnan model parameters may prove extremely useful for predicting the fate of metal contaminants.

Literature Cited

(1) Benedetti, M. F.; van Riemsdijk, W. H.; Koopal, L. K. *Environ. Sci. Technol.* **1996**, *30*, 1805−1813.

(2) Tombácz, E. *Soil Sci.* **1999**, *164*, 814−824.

(3) Christl, I.; Kretzschmar, R. *Environ. Sci. Technol.* **2001**, *35*, 2505−2511.

(4) Masini, J. C.; Abate, G.; Lima, E. C.; Hahn, L. C.; Nakurama, M. S.; Lichtig, J.; Nagatomy, H. R. *Anal. Chim. Acta* **1998**, *364*, 223−233.

(5) Cabaniss, S. E.; Zhou, Q.; Maurice, P. A.; Chin, Y.-P.; Aiken, G. R. *Environ. Sci. Technol.* **2000**, *34*, 1103−1109.

(6) Avena, M. J.; Koopal, L. K.; van Riemsdijk, W. H. *J. Colloid Interface Sci.* **1999**, *217*, 37−48.

(7) Christl, I.; Knicker, H.; Kögel-Knabner, I.; Kretzschmar, R. *European J. Soil Sci.* **2000**, *51*, 1−9.

(8) Růžička, J.; Hansen, E. H. *Anal. Chim. Acta* **1973**, *63*, 115−128.

(9) Avdeef, A.; Zabronsky, J.; Stuting, H. H. *Anal. Chem.* **1983**, *55*, 298−304.

(10) Yuchi, A.; Wada, H.; Nakagawa, G. *Anal. Chim. Acta* **1983**, *149*, 209−216.

(11) Milne, C. J.; Kinniburgh, D. G.; de Wit, J. C. M.; van Riemsdijk, W. H.; Koopal, L. K. *J. Colloid Interface Sci.* **1995**, *175*, 448−460.

(12) Benedetti, M. F.; Milne, C. J.; Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K. *Environ. Sci. Technol.* **1995**, *29*, 446−457.

(13) Kinniburgh, D. G.; Milne, C. J.; Venema, P. *Soil Sci. Soc. Am. J.* **1995**, *59*, 417−422.

(14) Keizer, M. G.; van Riemsdijk, W. H. *ECOSAT*; Department Environmental Sciences, Subdepartment Soil Sciences and Plant Nutrition, Wageningen Agricultural University: Wageningen, Netherlands, 1998.

(15) Baes, C. F.; Mesmer, R. E. *The hydrolysis of cations*; Wiley & Sons: New York, 1976.

(16) Smith, R. M.; Martell, A. E. *Critical Stability Constants Vol. 2: Amines*; Plenum Press: New York, 1990.

(17) Smith, R. M.; Martell, A. E. *Critical Stability Constants Vol. 4: Inorganic Complexes*; Plenum Press: New York, 1990.

(18) Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K.; Borkovec, M.; Benedetti, M. F.; Avena, M. J. *Colloids Surfaces A: Physicochem. Eng. Aspects* **1999**, *151*, 147−166.

(19) Christensen, J. B.; Tipping, E.; Kinniburgh, D. G.; Grøn, C.; Christensen, T. H. *Environ. Sci. Technol.* **1998**, *32*, 3346−3355.

(20) Pinheiro, J. P.; Mota, A. M.; Benedetti, M. F. *Environ. Sci. Technol.* **1999**, *33*, 3398−3404.

(21) Robertson, A. P.; Leckie, J. O. *Environ. Sci. Technol.* **1999**, *33*, 786−795.

(22) Pinheiro, J. P.; Mota, A. M.; Gonçalves, M. L. S. *Anal. Chim. Acta* **1994**, *284*, 525−537.

(23) Mota, A. M.; Rato, A.; Brazia, C.; Gonçalves, M. L. S. *Environ. Sci. Technol.* **1996**, *30*, 1970−1974.

(24) Saar, R. A.; Weber, J. H. *Geochim. Cosmochim. Acta* **1980**, *44*, 1381−1384.

(25) Hunter, R. J. *Foundations of Colloid Science. Vol. 1*; Oxford University Press: Oxford, 1986.

(26) Kretzschmar, R.; Sticher, H. *Environ. Sci. Technol.* **1997**, *31*, 3497−3504.

(27) Ricart, M.; Villaescusa, I.; de la Torre, F. *Reactive Functional Polym.* **1996**, *28*, 159−165.

(28) Murray, K.; Lindner, P. W. *J. Soil Sci.* **1983**, *34*, 511−523.

(29) Murray, K.; Lindner, P. W. *J. Soil Sci.* **1984**, 35, 217−222.

(30) Benedetti, M. F.; van Riemsdijk, W. H.; Koopal, L. K.; Kinniburgh, D. G.; Gooddy, D. C.; Milne, C. J. *Geochim. Cosmochim. Acta* **1996**, *60*, 2503−2513.