

Copper binding to soil fulvic and humic acids: NICA-Donnan modeling and conditional affinity spectra



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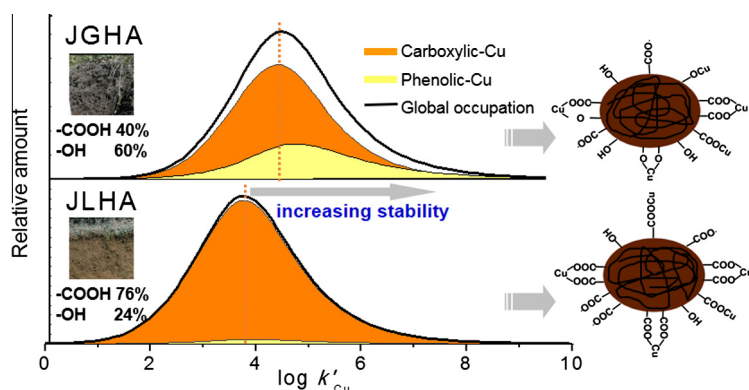
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GRAPHICAL ABSTRACT

Conditional affinity spectrum underlying NICA isotherm provides practical insight into the characteristics of metal ions binding to HS at constant environmental conditions. The distributions of binding affinity for bound species are estimated. Carboxylic groups make the main contributions to Cu binding to HS. For JLHA with relatively high amount of carboxylic groups, the occupation of phenolic type sites by Cu can be negligible. Phthalate-type structure is more probable for Cu binding to JLHA. There is a non-negligible amount of phenolic-Cu on JGHA, which increases the chance of forming more stable complexes.



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ABSTRACT

Binding of Cu(II) to soil fulvic acid (JGFA), soil humic acids (JGHA, JLHA), and lignite-based humic acid (PAHA) was investigated through NICA-Donnan modeling and conditional affinity spectrum (CAS). It is to extend the knowledge of copper binding by soil humic substances (HS) both in respect of enlarging the database of metal ion binding to HS and obtaining a good insight into Cu binding to the functional groups of FA and HA by using the NICA-Donnan model to unravel the intrinsic and conditional affinity spectra. Results showed that Cu binding to HS increased with increasing pH and decreasing ionic strength. The amount of Cu bound to the HAs was larger than the amount bound to JGFA. Milne's generic parameters did not provide satisfactory predictions for the present soil HS samples, while material-specific NICA-Donnan model parameters described and predicted Cu binding to the HS well. Both the 'low' and 'high' concentration fitting procedures indicated a substantial bidentate structure of the Cu complexes with HS. By means of CAS underlying NICA isotherm, which was scarcely used, the nature

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Speciation
Conditional affinity spectrum (CAS)

of the binding at different solution conditions for a given sample and the differences in binding mode were illustrated. It was indicated that carboxylic group played an indispensable role in Cu binding to HS in that the carboxylic CAS had stronger conditional affinity than the phenolic distribution due to its large degree of proton dissociation. The fact was especially true for JGFA and JLHA which contain much larger amount of carboxylic groups, and the occupation of phenolic sites by Cu was negligible. Comparable amounts of carboxylic and phenolic groups on PAHA and JGHA, increased the occupation of phenolic type sites by Cu. The binding strength of PAHA-Cu and JGHA-Cu was stronger than that of JGFA-Cu and JLHA-Cu. The presence of phenolic groups increased the chance of forming more stable complexes, such as the salicylate-Cu or catechol-Cu type structures.

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1. Introduction

Humic substances (HS) are natural soft particles that are ubiquitous in soils, sediments and waters [1,2]. Fulvic acid (FA) and humic acid (HA) are the predominant HS mass fractions, they are discriminated on the basis of their solubility at low pH: FA is soluble and composed of relatively small particles, HA is insoluble and composed of relatively large particles. HS are heterogeneous, poly-disperse dynamic associations of natural lower molar mass organic components stabilized by hydrophobic interactions and hydrogen bonds [3–5]. HS particles have a relatively open structure and contain numerous functional groups that in aqueous solutions become predominantly negatively charged due to proton dissociation and the dissociated groups can interact with other cations, such as metal ions. HS therefore affect metal ion bioavailability, mobility and toxicity in nature [6–8].

HS contain two major types of functional groups, carboxylic and phenolic, that are of particular importance for metal ion binding [9,10]. Although considerable literature is available, intuitive knowledge about the relative importance of these types of functional groups for the complexation of metal ions is ambiguous. Some authors have emphasized that at natural pH values the carboxylic type groups are stronger dissociated than the phenolic type groups and that therefore the first should dominate metal ion binding to HS [11,12]. However, in other studies it has been suggested that phenolic type sites also play a significant role in metal ion binding or even make a larger contribution than the carboxylic type sites [13–15]. Clearly the answer is not simple, but assessment of the stability or affinity constants of metal ion complexes with the functional groups of HS is necessary to predict the metal ion complexation properties of HS and to explain the transport of metal ions in the environment [14]. Due to the heterogeneity of both the carboxylic and phenolic groups and the variably charged nature of the HS particles the assessment of the 'intrinsic' affinity constants (independent of the solution composition) of metal ions with the functional groups can only be achieved through sophisticated speciation modeling.

In the past decades, there has been considerable progress in the development of chemical speciation models that are able to describe metal ion binding to HS over a wide range of metal concentrations and solution conditions on the basis of 'intrinsic' affinity constants. The most versatile models are the NICA (non-ideal competitive adsorption)–Donnan model [16] and Tipping's Model V/VI/VII [17,18]. In these models both site heterogeneity, metal/proton and metal/metal competition and electrostatic interactions are taken explicitly into account, but the way these factors are modeled is somewhat different. Here we focus on the NICA–Donnan model that uses a continuous affinity distribution for both the carboxylic (low proton affinity) and phenolic (high proton affinity) type sites and involves the Donnan model to account for the generic electrostatic interactions. An advantage of the NICA–Donnan model over Tipping-type models is that it can be applied

relatively easily to given data set. Once the NICA–Donnan model parameters for a HS–metal ion system are known, the speciation of the metal ion (carboxylic-type complexes, phenolic-type complexes, electrostatically bound metal ions in the Donnan phase, and the free metal ions in bulk solution) can be calculated as a function of the metal ion concentration, pH, and ionic strength [8,19]. In principle this information is sufficient for the description of the metal ion binding, but the values of the intrinsic affinity constants and heterogeneity parameters cannot be interpreted intuitively in terms of 'conditional' binding affinities and their heterogeneity at given solution conditions.

The conditional affinity is a useful parameter in the case of multicomponent binding. When in a multicomponent system the concentration of all solutes is kept constant, except component *i*, the binding of component *i* can be described as pseudo-monocomponent binding. The resulting conditional affinity is a function of the conditions, but at the given conditions and ideal binding behavior it is a constant. In the case of site heterogeneity or other non-idealities a distribution of conditional affinities results. In general, the transformation of a competitive binding isotherm at given conditions in a 'conditional affinity spectrum' or CAS provides a straightforward methodology to interpret isotherms in terms of pseudo-monocomponent affinities [20,21]. The CAS methodology has been applied to simple competitive systems [22,23] as well as to complex natural media [20,24]. The CAS underlying the NICA isotherm has also been developed [25] and provides practical insight into the characteristics of metal ions binding to HS at constant environmental conditions.

Besides, the chemical heterogeneity within a HS, also different HS have different ion binding properties [3,26] that result from their differences in amount of functional groups and relative presence of carboxylic and phenolic type groups. Based on existing data on proton and metal ion binding to both FA and HA, Milne et al. [27,28] have derived generic NICA–Donnan parameter sets (comprising generic intrinsic stability constants, non-ideality parameters and heterogeneity parameters) that define 'average' or generic-FA and 'average' or generic-HA. These parameter sets can be used to discuss FA and HA metal ion binding in general and in specific cases when no or little information is present about a given FA or HA sample. However, the description of the metal ion binding behavior of a specific FA or HA with these generic parameter sets is not always satisfactory [29,30]. Therefore, it is still useful to extend the database of metal ion binding to different HA and FA samples, so that in the long run the generic parameter sets can be revised and improved. When specific results for FA or HA cannot be described well with the relevant generic parameter sets, material-specific NICA–Donnan model parameters are required to obtain a good insight in the role of each of the functional groups of the sample in the metal ion binding.

The present study aims to extend our knowledge of copper binding by soil HS both with respect to enlarge the database of Cu²⁺ binding to soil FA and HA and to provide further insight into

Cu binding to the functional groups of FA and HA by using the NICA-Donnan model to unravel the intrinsic and conditional affinity spectra. The soil HS have been extracted from different origins in China; the proton binding results of these samples have been obtained previously [31] and the Cu binding is measured as a function of pH and ionic strength and the analysis of the results by the NICA-Donnan model provides the material-specific parameters. The present results for the soil HAs are compared with two references: Milne's generic-HA [28] and purified Aldrich humic acid (PAHA), a much studied HA. The results for the soil FA are compared with Milne's generic-FA and with HA from the same location. The comparison is based on both the calculated Cu binding isotherms and the Cu speciation at different pH and ionic strength. Furthermore, the conditional affinity spectra underlying NICA isotherm are used to illustrate the nature of the binding at different solution conditions for a given sample and to unravel the differences in binding mode.

2. Materials and methods

2.1. Preparation of FA and HA

The HS samples were extracted from (1) a mountainous meadow soil (semi-hydromorphic soil) of Jiugong Mountain, Hubei Province, China (N 29°27', E 114°42') located in the subtropical monsoon climate zone and (2) the upper horizon of a brown soil (Alfisol) in Tonghua, Jilin Province, China (N 41°30', E 125°55') with a temperate monsoon climate. The FA and HAs were isolated and purified following the standard procedure of the International Humic Substances Society [32]. The purified samples will be denoted as JGFA and JGHA for those extracted from the site in Jiugong, and JLHA for that from Jilin. As reference PAHA, Aldrich humic acid (CAS 6813-04-4) purified using the method described by Vermeer et al. [33] was included. Stock HS solutions of 2 g L⁻¹ were prepared at pH ≥ 10 with KOH, equilibrated for 24 h to dissolve the HS completely [34] and stored in the dark at 4 °C. Characterization of the samples (JGFA, JGHA, JLHA and PAHA), their proton binding and the NICA-Donnan modeling of the proton results have been described in detail in previous papers [26,31,35].

2.2. Copper binding to HS

The binding of Cu to JGFA, PAHA, JGHA, and JLHA was measured at three pH values (4.0, 5.0 and 6.0) and two KNO₃ concentrations (0.01 M and 0.1 M) using the Cu titration method in combination with the pH-stat method [3]. Prior to the Cu titration the HS solution was purged with N₂ gas to remove possible CO₂ and then lowered pH to the desired value by HNO₃ addition, keeping constant for 10 h.

Titrations were performed at 25 ± 0.1 °C under nitrogen gas atmosphere using an automated titration setup (Metrohm, 836 Titrando). After each step the equilibrium Cu²⁺ concentration was measured potentiometrically with a Cu ion-selective electrode (Cu-ISE) (Metrohm, 6.0502.140) in combination with an Ag/AgCl reference electrode (Metrohm, 6.0726.107). To avoid Cl⁻ leakage in the titration cell the reference electrode was connected to the HS solution through an electrolyte bridge containing a KNO₃ solution of similar ionic strength as the HS solution. The Cu²⁺/KNO₃/Ag/AgCl cell EMF was calibrated prior to each titration. A further description of the calibration of Cu-ref electrode system can be found in the Supporting Information (SI). During the Cu titration the pH in the solution was monitored and kept at its initial value using an automated pH-stat technique. After each Cu addition, the HS solution was stirred for 2 min and the electrode readings were made. When after an equilibration time >6 min the

Cu-potential drift was <0.2 mV min⁻¹ or after a maximum waiting time of 15 min the Cu-potential was recorded. Each titration was stopped when Cu (hydr-)oxide precipitation could occur according to calculations using the chemical speciation program ECOSAT (Equilibrium Calculation of Speciation and Transport) [36]. The amount of Cu sorbed to HS was calculated by subtracting the sum of aqueous copper species from the known total amount of Cu in solution. The Cu binding data were fitted to the NICA-Donnan model using the computer codes ECOSAT [36] extended with the code FIT [37].

3. Theoretical background

3.1. NICA-Donnan model

Cation binding to HS is assumed to occur through specific interactions at the available binding sites and by nonspecific (electrostatic) binding to any residual negative charge. The NICA-Donnan model for HS accounts for two types of binding sites, often assumed to correspond to carboxylic (type 1) and phenolic (type 2) sites. The NICA part of the model takes into account the chemical (specific) heterogeneity of both types of functional groups in terms of intrinsic affinities for protons or metal ions. The Donnan part describes the non-specific electrostatic interactions and accounts for diffusely bound cations. The NICA equation for the cation-specific binding to each of the site types is given by Eq. (1) [38,39]:

$$\theta_{i,s} = \frac{(\bar{k}_{i,s} c_{D,i})^{n_{i,s}} \left(\sum_i (\bar{k}_{i,s} c_{D,i})^{n_{i,s}} \right)^{p_s}}{\sum_i (\bar{k}_{i,s} c_{D,i})^{n_{i,s}} + \left(\sum_i (\bar{k}_{i,s} c_{D,i})^{n_{i,s}} \right)^{p_s}} \quad (1)$$

where subscript *s* refers to the modal distribution (1: carboxylic and 2: phenolic); θ_i is the fraction of sites occupied by species *i*; \bar{k}_i is the median value of the intrinsic affinity distribution for species *i*; $c_{D,i}$ is the concentration of species *i* in the Donnan phase. The ion-specific stoichiometry (non-ideality) n_i ($0 < n_i \leq 1$), varies from ion to ion and the heterogeneity parameter p ($0 < p \leq 1$) is related to the width of the intrinsic affinity distribution. The intrinsic affinity distribution reflects the chemical heterogeneity of the given site type and is a so-called Sips-distribution [40,41]:

$$p_s(\log k_i; c_H = 0) = \frac{\ln(10)}{\pi} \frac{\sin(\pi n_{i,s} p_s)}{(\bar{k}_{i,s}/k_i)^{-n_{i,s} p_s} + (\bar{k}_{i,s}/k_i)^{n_{i,s} p_s} + 2 \cos(\pi n_{i,s} p_s)} \quad (2)$$

where k_i is the intrinsic affinity for a group of equal affinity sites. The total amount of specifically bound ion *i*, $Q_{i,t}$, to site type 1 and 2 is given by

$$Q_{i,t} = \theta_{i,1} \left(\frac{n_{i,1}}{n_{H,1}} \right) Q_{\max H,1} + \theta_{i,2} \left(\frac{n_{i,2}}{n_{H,2}} \right) Q_{\max H,2} \quad (3)$$

where $Q_{\max H,s}$ is the maximum site density for each type site *s*, as observed with proton adsorption (subscript H refers to proton), i.e., the proton is used as reference cation. The factor n_i/n_H in Eq. (3) denotes the average stoichiometry of metal ion *i* bound to sites 1 or 2 using the average proton stoichiometry as reference. The product $(n_i/n_H) \cdot Q_{\max H}$ expresses that the maximum binding of *i* may be different from that of H.

The Donnan model assumes that the HS particles form a gel-like phase with a volume V_D , the Donnan volume [42], in which the net charge of the humic particles, q , is completely neutralized by counterion sorption and coion exclusion [19]:

$$q/V_D + \sum z_j (c_{D,j} - c_j) = 0 \quad (4)$$

where z_j represents the charge of ion *j* (including sign), c_j is the concentration of ion *j* in the bulk solution and $c_{D,j}$ is the concentration

of j in the Donnan volume. The summation includes all electrolyte ions (including i). The concentration $c_{D,j}$ is related to c_j by a Boltzmann factor including the Donnan potential, ψ_D :

$$c_{D,j} = c_j \exp\left(-\frac{z_j F \psi_D}{RT}\right) \quad (5)$$

where F is the Faraday constant, R the gas constant and T the absolute temperature. The Donnan volume (V_D) is a function of the ionic strength and calculated with the empirical relationship [42]:

$$\log V_D = b(\log I - 1) - 1 \quad (6)$$

where I is the ionic strength and b is a fitting parameter. The NICA model and the Donnan model are interrelated since the Donnan phase concentrations, $c_{D,j}$, of the various components are also used to calculate the ion specific binding (Eq. (1)). Therefore, the NICA-Donnan equation can only be solved by iteration [28].

3.2. Conditional affinity spectra

Conditional affinity spectra describe the effective distribution of affinities or binding energies experienced by one complexing agent at a fixed concentration of the rest [25]. For the HS/H⁺-Cu²⁺ competitive system, the CAS provides the relative amount of HS binding sites of each affinity that are available for Cu²⁺ under the restriction of a fixed H⁺ concentration ($c_H = cnt$). Detailed analytical expressions of the CAS underlying the NICA isotherm have been reported [20,25]. By fixing c_H , the two-component isotherm becomes pseudo mono-component, and the CAS mono-dimensional. For the monomodal case, the analytical expression for the CAS becomes:

$$p_s(\log k'_{Cu}; c_H = cnt) = \frac{\ln(10)}{\pi} \left(\frac{\frac{n_{Cu,s}}{n_{H,s}} Q_{\max H,s}}{\frac{n_{Cu,1}}{n_{H,1}} Q_{\max H,1} + \frac{n_{Cu,2}}{n_{H,2}} Q_{\max H,2}} \right) \times \left(\frac{(\bar{k}_{Cu,s}/k'_{Cu})^{n_{Cu,s}} M_s^{p_s-1}}{1 + M_s^{2p_s} + 2M_s^{p_s} \cos(p_s \phi_s)} [\sin(\pi n_{Cu,s} - (1 - p_s)\phi_s) + M_s^{p_s} \sin(\pi n_{Cu,s} - \phi_s)] \right) \quad (7)$$

where k'_{Cu} ($c_H = cnt$) is the conditional Cu affinity for a site,

$$M_s = \left[(\bar{k}_{Cu,s} c_{D,H})^{2n_{H,s}} + (\bar{k}_{Cu,s}/k'_{Cu})^{2n_{Cu,s}} + 2(\bar{k}_{H,s} c_{D,H})^{n_{H,s}} (\bar{k}_{Cu,s}/k'_{Cu})^{n_{Cu,s}} \cos(\pi n_{Cu,s}) \right]^{1/2} \quad (8)$$

and

$$\cos(\phi_s) = \frac{(\bar{k}_{H,s} c_{D,H})^{n_{H,s}} + (\bar{k}_{Cu,s}/k'_{Cu})^{n_{Cu,s}} \cos(\pi n_{Cu,s})}{M_s} \quad (9)$$

where $\log \bar{k}_H$ also represents the mean energy to be expended by Cu in removing proton. To better understand the contribution of each monomodal to the overall Cu binding, the weighting factor of $(n_{Cu,s}/n_{H,s})Q_{\max H,s}/((n_{Cu,1}/n_{H,1})Q_{\max H,1} + (n_{Cu,2}/n_{H,2})Q_{\max H,2})$ of carboxylic and phenolic distributions is included in Eq. (7). The direct addition of each modal distribution ($s = 1, 2$) leads to the corresponding global or bimodal CAS. To concisely characterize a given CAS, the average affinity for Cu (at $c_H = cnt$) and the variance are required. The average affinity is conveyed by the first moment of the distribution:

$$\langle \log k'_{Cu} \rangle = \int_{-\infty}^{\infty} \log k'_{Cu} p(\log k'_{Cu}; c_H = cnt) d \log k'_{Cu} \quad (10)$$

where the symbol $\langle \rangle$ is used to denote the average over the conditional affinity spectrum.

The variance, which provides information on the heterogeneity of the binding is given by the second central moment of the distribution:

$$\sigma_{Cu}^2 = \langle \log k'_{Cu} \rangle^2 - \langle \log k'_{Cu} \rangle^2 \quad (11)$$

where

$$\langle \log k'_{Cu} \rangle = \int_{-\infty}^{\infty} \log k'_{Cu} p(\log k'_{Cu}; c_H = cnt) d \log k'_{Cu} \quad (12)$$

The CAS provides the relative amount of binding sites of each affinity that are available for a given ion. Despite their usefulness, the average values of the CAS do not necessarily reflect the energy exchanged in the sites that are actually involved in the binding [20]. The distribution of sites occupied with Cu at a given equilibrium concentration of Cu²⁺, can be calculated from the CAS as follows:

$$p_{occ}(\log k'_{Cu}, c_{D,Cu}) = p(\log k'_{Cu}; c_H = cnt) \frac{k'_{Cu} c_{D,Cu}}{1 + k'_{Cu} c_{D,Cu}} \quad (13)$$

where $c_{D,H}$ and $c_{D,Cu}$ are the H and Cu concentration in the Donnan volume, respectively. The Cu concentration in the Donnan phase is related through Eq. (5) to the equilibrium Cu concentration in bulk solution. It should be noticed that, in general, the Cu concentrations in the Donnan phase are significantly higher than in bulk solution. The average conditional affinity of Cu occupied (*occ*) sites should be calculated with Eq. (14):

$$\langle \log k'_{Cu} \rangle_{occ} = \int_{-\infty}^{\infty} \log k'_{Cu} p_{occ}(\log k'_{Cu}, c_{D,Cu}) d \log k'_{Cu} \quad (c_H, c_{Cu} = cnt) \quad (14)$$

4. Results and discussion

4.1. Binding isotherms

The binding isotherms for Cu to JGFA, PAHA, JGHA and JLHA are presented in Fig. 1. All binding isotherms plotted on a double logarithmic plot are nearly linear at low Cu concentrations, the slope of which is close to 0.5. At high Cu concentrations, the isotherms become curved indicating the beginning of saturation with respect to Cu binding. An increase in Cu binding with increasing pH is observed. The ionic strength effect is measured at pH 6.0. The amount of Cu binding increases with decreasing ionic strength due to the lesser screening of the electrostatic attraction between Cu and the negatively charged functional groups.

For all Cu concentrations, the amount of Cu bound by the HAs is greater than the amount bound by the JGFA at each pH values although the sites density of acidic functional groups of JGFA is considerably larger than that of the HAs. Though JGFA and JGHA were extracted from the same location, their Cu binding behavior is different. The elemental analysis in our previous study [31] indicated that the aliphaticity of JGFA is larger than that of JGHA and the other HAs. As revealed by Christl et al. [43,44], compared to FA a greater percentage of carboxylic groups in HA is linked to aromatic rings than to aliphatic chains. This leads for HAs to a higher probability of adjacent carboxylic and phenolic groups (aromatic) capable of forming strong complexes with Cu. Gondar et al. [45] also observed significant differences on Cu binding to FA and HA at low Cu concentrations due to this structural effect. In their study there were almost no differences at high concentrations between binding curves corresponding to HA and FA from the same peat sample, highlighting that the presence of more binding sites in FA could compensate for the effect of structural differences. However, in the present study, the maximum binding capacity of FA is smaller than that of the HAs samples, suggesting that the structural configuration of binding sites instead of the total amount of acidic functional groups has a strong influence on the Cu binding behavior to HS.

The comparison of the binding isotherms of Cu to the different HA samples reveals that the shapes of the binding isotherms and

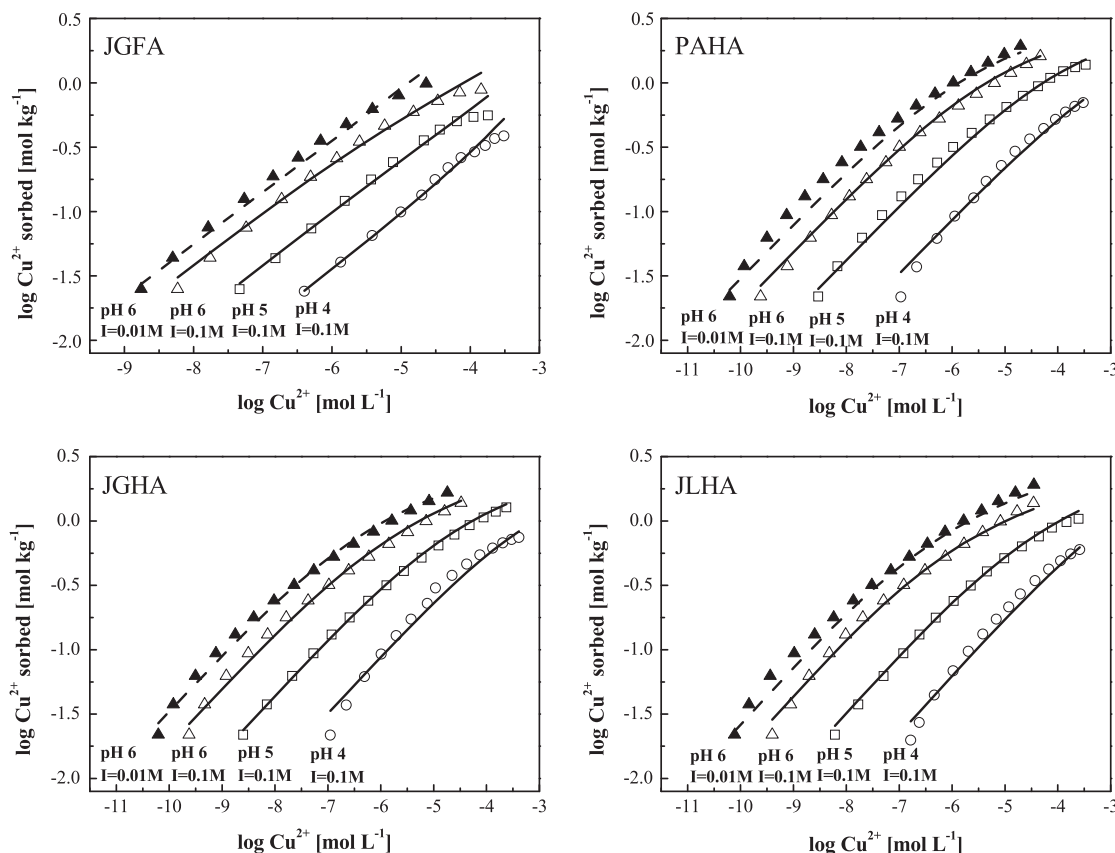


Fig. 1. Copper binding to FA and HAs at pH 4.0, 5.0, and 6.0 in 0.01 and 0.1 M KNO_3 plotted against the equilibrium Cu^{2+} concentration. Symbols represent the experimental data. Curves represent results calculated with the NICA-Donnan model using the material-specific parameters obtained from the double logarithmic optimization.

the amount of Cu bound by PAHA and soil HAs at pH 6.0 are fairly similar, independent of the origins from which the samples were extracted. At pH 4.0 and 5.0 the amounts of Cu bound to PAHA and JGHA are nearly identical, but slightly larger than that to JLHA. The difference becomes smaller at high Cu concentrations (Fig. 1). These results point to a somewhat higher affinity of Cu for PAHA and JGHA than for JLHA. The observed distinction may be attributed to different chemical composition, functional group contents and particle size of HAs.

4.2. NICA-Donnan modeling

The double logarithmic Cu binding isotherms to JGFA and HAs were fitted with the NICA-Donnan model. The optimized parameters for Cu binding, together with the parameters for proton binding, are shown in Table 1. The goodness of fit of the model is good, the R^2 values are greater than 0.986. See also in Fig. 1, the NICA-Donnan model calculations (solid lines) agree well with the experimental data (symbols) for Cu binding, but high Cu loadings of FA were not fitted accurately. To test whether the NICA-Donnan model calculates the effect of ionic strength on Cu binding according to experimental observations, the parameters were then used to predict the Cu binding to HS in a 0.01 M KNO_3 at pH 6.0. The calculations (dashed lines) excellently correspond to the experimental data (symbols) on Cu binding in 0.01 M KNO_3 . The obtained NICA-Donnan parameters for Cu binding to HS are sample specific. The binding constants and the heterogeneity parameters are partly in agreement with those reported in previous studies [3,19,27,28,45–47]. The values of $n_{\text{Cu}}/n_{\text{H}}$ represent the averaged stoichiometry over the pH and $\log [\text{Cu}^{2+}]$ range. The closer $n_{\text{Cu}}/n_{\text{H}}$ is to 1, the more likely the metal ion is bound as a monodentate

complex, the closer it is to 0.5 the more likely the binding is bidentate [48]. As can be seen in Table 1, the $n_{\text{Cu}}/n_{\text{H}}$ ratios are relatively close to 0.5 for the two types of groups of both FA and HAs, i.e., on average 0.57–0.67 and 0.46–0.69 molecules of Cu are bound to one carboxylic and phenolic type site, respectively, suggesting significant bidentate complexation.

By using double logarithmic Cu binding isotherms to fit the data a relatively high weight is given to low concentrations [26]. More accurate information at high Cu concentrations is obtained when the Cu-isotherms plotted as the $[\text{Cu}^{2+} \text{ bound}]$ vs. $\log [\text{Cu}^{2+}]$ are fitted to the model (keeping the proton binding parameters the same). Comparison of the two fits reveals difference in binding stoichiometry at low and high bound amounts most clearly. The results in the form of double logarithmic plots are included in the SI (Fig. S2). The new fitting method describes the Cu binding to HS well at high Cu concentrations, but less good at low Cu concentrations, especially for JGFA. The obtained model parameters are also collected in Table 1, they differ somewhat from the values obtained with the double logarithmic plots. The newly observed $n_{\text{Cu}}/n_{\text{H}}$ ratios of HAs range from 0.46 to 0.86, indicating that copper is bound by HS as monodentate or bidentate complexes at high Cu loadings. Bidentate complexes are more stable and are expected to form in a high proportion. The new $n_{\text{Cu}}/n_{\text{H}}$ ratios of HAs for phenolic groups are somewhat larger than those obtained from the double logarithmic optimization, implying a higher degree of monodentate complexation with the phenolic type of sites with increasing Cu loading. For JGFA, the values of $n_{\text{Cu}}/n_{\text{H}}$ ratios are about 0.5 in both fitting modes and hence bidentate complexes should be the dominating structures at both high and low Cu loadings. Consequently, both the low and high concentration fitting procedures lead to values of $n_{\text{Cu}}/n_{\text{H}}$ somewhat lower than unity,

Table 1
NICA-Donnan model parameters obtained from 'low' and 'high' concentration fitting procedures.

	JGFA		GFA		PAHA		JGHA		JLHA		GHA
	Low	High	Low	High	Low	High	Low	High	Low	High	Low
b^*	0.81	0.81	0.57	0.60	0.60	0.48	0.48	0.47	0.47	0.47	0.49
$Q_{\max H,1}^*$	8.72	8.72	5.88	2.97	2.97	2.35	2.35	4.46	4.46	4.46	3.15
p_1	0.72	0.76	0.59	0.90	0.92	0.80	0.92	0.53	0.77	0.77	0.62
$\log \bar{k}_{H,1}^*$	2.18	2.18	2.34	3.52	3.52	3.27	3.27	2.95	2.95	2.95	2.93
n_{H1}	0.74	0.70	0.66	0.72	0.71	0.87	0.76	0.78	0.53	0.53	0.81
$\log \bar{k}_{Cu,1}$	-1.39	-2.13	0.26	2.37	2.51	2.52	2.54	1.06	0.40	0.40	2.23
n_{Cu1}	0.42	0.34	0.53	0.48	0.44	0.54	0.45	0.49	0.38	0.38	0.56
n_{Cu1}/n_{H1}	0.57	0.49	0.80	0.67	0.62	0.62	0.59	0.63	0.72	0.72	0.69
$Q_{\max H,2}^*$	3.90	3.90	1.86	2.86	2.86	3.48	3.48	1.38	1.38	1.38	2.55
p_2	0.66	0.71	0.70	0.44	0.38	0.53	0.49	0.82	0.83	0.83	0.41
$\log \bar{k}_{H,2}^*$	8.19	8.19	8.60	7.99	7.99	8.33	8.33	7.52	7.52	7.52	8.00
n_{H2}	0.89	0.83	0.76	0.59	0.68	0.59	0.63	0.82	0.81	0.81	0.63
$\log \bar{k}_{Cu,2}$	6.77	7.54	8.26	6.42	6.00	7.27	5.75	6.68	7.24	7.24	6.85
n_{Cu2}	0.41	0.30	0.36	0.41	0.54	0.35	0.54	0.38	0.45	0.45	0.34
n_{Cu2}/n_{H2}	0.46	0.36	0.47	0.69	0.79	0.59	0.86	0.46	0.56	0.56	0.54
R^2	0.986	0.969	0.90	0.996	0.998	0.994	0.997	0.993	0.993	0.993	0.90

The parameters for proton binding (marked with *) were taken from Tan et al. [31]. b is Donnan volume parameter. $Q_{\max H}$ is sites density (mol kg⁻¹). \bar{k}_i is the median value of the intrinsic affinity distribution for species i . p is a parameter reflecting the intrinsic heterogeneity of HS. n_i is the non-ideal ion-specific parameter of species i . The indications 'low' and 'high' in the second row represent the type of fitting of the model parameters: 'low' implies parameters obtained from the fitting log [Cu bound] vs. log [Cu²⁺] (most weight to low Cu loading or Cu concentration) and 'high' parameters from fitting [Cu bound] vs. log [Cu²⁺] (most weight to high Cu loading or Cu concentration). Note that the parameters obtained at high concentration are mainly used to discuss the changes in Cu stoichiometry along the isotherms. For all CAS calculations the low concentration parameters are used, as these are more environmentally relevant. The parameters of GFA and GHA were taken from Milne et al. [28].

which reflects a substantial bidentate structures of the Cu complexes with HS.

4.3. Copper speciation

The total speciation of Cu is mainly composed of four contributions: the dissolved ion [Cu²⁺](aq), Cu bound to carboxylic type sites (Q_1), Cu bound to the phenolic type sites (Q_2) and Cu located in the Donnan phase (Donnan). In the range of conditions studied, other species can be neglected compared to the large amount of free Cu²⁺ (see Fig. S1). The estimated Cu speciation as a function of log [Cu²⁺]_{total} in presence of 0.8 g L⁻¹ HS in 0.1 M KNO₃ at pH 4.0, 5.0, 6.0 is shown in Fig. 2. The double logarithmic fitted parameters (low Cu concentrations) are used for the calculations as these are most environmentally relevant. The Cu species are all expressed in mol L⁻¹ for easy comparison. The amount of [Cu²⁺](aq) is smaller than that bound to HS for the whole range of total Cu concentrations. At low total Cu concentrations, almost all Cu binds to HS; at high total Cu concentrations, the higher affinity sites become saturated and dissolved Cu becomes increasingly important. The amount of Cu bound to carboxylic type sites plus phenolic type sites is much greater than that in Donnan phase, indicating that most Cu bound to HS is by specific interaction.

For JGFA, the amount of Cu bound to phenolic type sites is smaller by about 1–2 orders of magnitude than to carboxylic type sites. It means that Cu binding is controlled by carboxylic type sites. The FA phenolic sites are hardly significant and become less important than the Donnan phase at pH 4.0 and 5.0 at high total Cu concentrations. The Cu speciation of JLHA is largely similar to that of JGFA. For PAHA, carboxylic type sites are more important than phenolic type sites in Cu binding. The Donnan contribution to Cu binding to PAHA is negligible. For JGHA, both carboxylic and phenolic sites control the Cu binding. The phenolic sites are more important at low total Cu concentrations, whereas the carboxylic sites dominate at relatively high total Cu concentrations. Compared with the carboxylic contents of JGHA (2.35 mol kg⁻¹) and PAHA (2.97 mol kg⁻¹), JGFA (8.72 mol kg⁻¹) and JLHA (4.46 mol kg⁻¹) contain much more carboxylic sites. This largely explains the dominance of the carboxylic-Cu complexation in the case of JGFA and JLHA. Moreover, the carboxylic sites deprotonate relatively easily

and this contributes to the negative charge of the particles. For JGFA and JLHA with a relatively high carboxylic site density this leads to relatively high amounts of Cu being bound in the Donnan phase. Such differences will be relevant in the environment because ions bound in the Donnan phase behave quite differently with respect to competition with cations (such as sodium and calcium), than ions specifically bound to HS [29].

It is obvious that Cu speciation largely depends on pH. The pH-dependence of the speciation follows the order: Cu in Donnan volume > phenolic-Cu > carboxylic-Cu. The amount of phenolic-Cu increases with increasing pH, while the amount of Cu in Donnan volume decreases with increasing pH. The effect of pH on carboxylic-Cu is limited except for Cu binding to JGHA. The Donnan phase is not significant at trace Cu concentrations. With increasing Cu concentration, the Donnan phase becomes increasingly significant.

4.4. Comparison with predictions using Milne's NICA-Donnan parameters

The generic NICA-Donnan parameters for both 'average-FA' (GFA) and 'average-HA' (GHA) for Cu were based on eleven datasets GFA-Cu (FCu-01-11) and eight datasets GHA-Cu (HCu-01, 03-06, 08-10) [28]. About half of the datasets were soil HS extracted from European and American soils and the differences between different samples were considerable. The generic parameters are also included in Table 1. Comparison of the parameter values for generic HS with the comparable parameters (low concentration fit) for the present samples (Table 1) shows that the density of both carboxylic and phenolic groups ($Q_{\max H,1}$ and $Q_{\max H,2}$) on JGFA is larger than that on GFA and the $\log \bar{k}_H$ and $\log \bar{k}_{Cu}$ values of JGFA are lower than that of generic FA. In general, the parameter values for GHA are in the range of those obtained for PAHA, JGHA and JLHA.

The experimental binding isotherms and the predictions based on the generic NICA-Donnan parameters at pH 4.0, 5.0 and 6.0 in 0.1 M and 0.01 M KNO₃ are depicted in Fig. 3a (FA) and b (HA). The generic FA parameters overestimate the Cu binding to JGFA considerably. A similar trend is observed for HA, but the differences are somewhat smaller and at pH 5.0 and pH 6.0 the GHA-

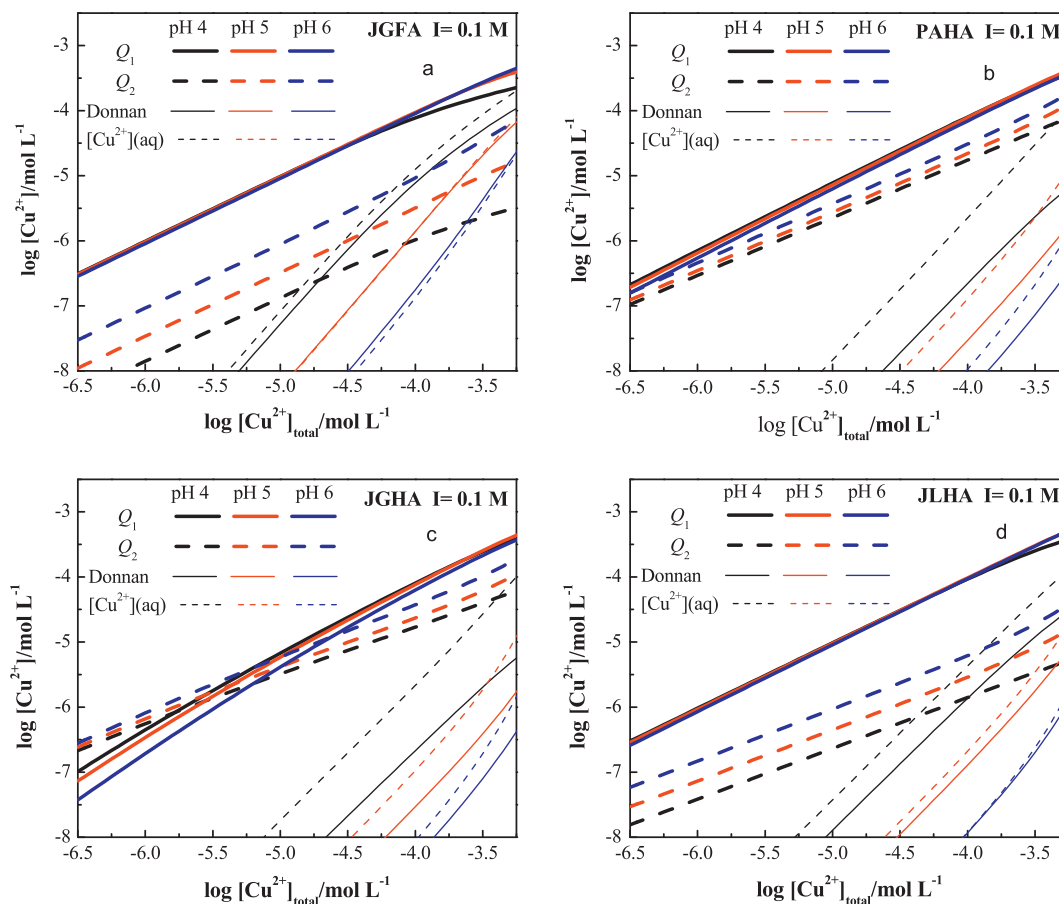


Fig. 2. Copper speciation in presence of HS as a function of total Cu^{2+} concentration with the double logarithmic fitted parameters at pH 4.0, 5.0 and 6.0 in 0.1 M KNO_3 . Thin dashed lines: aqueous Cu^{2+} concentration; thick solid lines: Cu^{2+} bound to carboxylic sites (Q_1); thick dashed lines: Cu^{2+} bound to phenolic sites (Q_2); thin solid line: electrostatically bound Cu^{2+} in the Donnan phase.

Cu results correspond relatively well to the experiment data at high bound amounts.

Copper speciation for GFA (Fig. 3c) and GHA (Fig. 3d) in 0.1 M KNO_3 solution and pH 4.0–6.0 as calculated with the generic NICA-Donnan parameters can be compared with Fig. 2a (JGFA) and b (PAHA), c (JGHA) and d (JLHA). For FA the comparison indicates that the amount of Cu binding to carboxylic groups of JGFA is comparable to that of GFA, but that the role of the phenolic sites is somewhat more prominent for GFA and that of the Donnan phase is less important. The copper speciation for GHA is closest to that of PAHA, for JGHA the role of phenolic sites is more prominent and for JLHA the phenolic sites are less prominent. Overall, the carboxylic-Cu is still the most important species.

4.5. Copper binding visualized with CAS

4.5.1. The CAS associated with NICA isotherms at different pH

The CAS allows an alternative representation and interpretation of specific binding properties of Cu to the HS samples. The calculations of the CAS results are based on the NICA parameters fitted using the double logarithmic plots, see the columns ‘low’ in Table 1, together with the estimated Donnan concentrations $c_{D,i}$. The bimodal CAS of HS-Cu binding at the three pH values in 0.1 M KNO_3 was computed for the different samples. The selected total Cu^{2+} concentration is $1 \times 10^{-4} \text{ mol L}^{-1}$ (the equilibrium Cu^{2+} concentration ranges from $1.3 \times 10^{-5} \text{ mol L}^{-1}$ to $8.8 \times 10^{-9} \text{ mol L}^{-1}$). Results for a lower total Cu^{2+} concentration of $1 \times 10^{-6} \text{ mol L}^{-1}$ are present in SI. The CASs at $10^{-4} \text{ mol L}^{-1}$ Cu are presented in Fig. 4 for JGFA

and the three HAs; for each pH the carboxylic and phenolic part within the global CAS is depicted. Note that the area under each modal distribution at a given pH reflects the contribution of the carboxylic and phenolic sites to the overall Cu binding, i.e., the direct addition of the two curves leads to the bimodal CAS [24].

The CASs for different pH values show in a direct way the influence of pH on the effective Cu binding affinities of the carboxylic (solid lines) and phenolic (dashed lines) type sites. The CAS shifts with increasing pH toward higher conditional affinities for both carboxylic sites and phenolic sites, but the shift of the phenolic sites is larger. In general, this behavior is due to the decreasing energy expended in extracting protons for Cu binding. The fact that the shift of phenolic distribution is larger than that of the carboxylic distribution indicates that change in proton dissociation in the given pH range is larger for the phenolic sites than for the carboxylic sites. This is due to the relatively high proton occupation and a larger ion exchange energy required to remove protons of phenolic sites at the studied pHs [20,22]. This result agrees with the speciation calculations that pH influences the amount of phenolic-Cu more strongly than carboxylic-Cu at $[\text{Cu}^{2+}]_{\text{total}} = 10^{-4} \text{ mol L}^{-1}$. Furthermore, the shift of the phenolic distribution is so considerable that it becomes weaker than the carboxylic distribution for all samples. This behavior is the opposite of what would be expected from the intrinsic affinities (green lines in Fig. 4), because these affinities only reflect Cu binding to sites that are dissociated.

In the above the ‘‘elementary’’ CASs for both carboxylic and phenolic sites are depicted. As the monomodal CASs overlap to a great extent at pH 4.0–6.0, the bimodal CAS evolves from a double

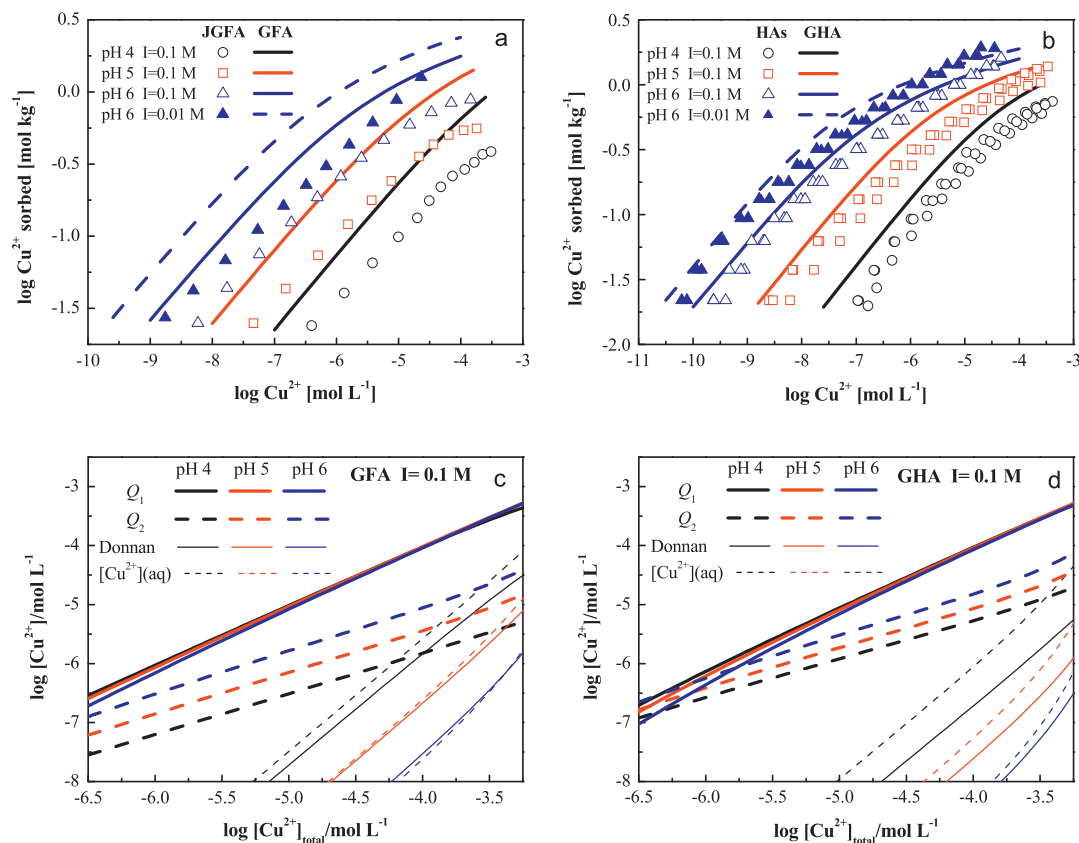


Fig. 3. Predictions based on the generic NICA-Donnan parameters. Panel a and b: comparison with the experimental Cu binding isotherms (symbols) for JGFA (panel a) and for PAHA, JGHA and JLHA (panel b) at pH 4.0, 5.0 and 6.0 in 0.01 and 0.1 M KNO₃. With the symbols no distinction has been made between the different HAs. The calculated Cu speciation based on the generic parameters is shown in panel (c) for GFA and in panel (d) for GHA.

peaked shape at pH 4.0 to a single peak at pH 6.0. For more precise comparison Fig. S3 in SI should be consulted. The shapes of the bimodal CAS imply a strong competition between carboxylic sites and phenolic sites for Cu, especially at pH 6.0. This conclusion corresponds to the fact that there is a smaller discrepancy between the role of carboxylic-Cu and phenolic-Cu at higher pH for the total Cu concentration of 10^{-4} mol L⁻¹ in Fig. 2.

4.5.2. The distribution of occupied sites

The distribution of occupied sites provides the relative abundance of binding sites of each affinity that are involved in the binding [20], which can be used to analyze where Cu ions bind for a given pH and Cu concentration. The solid lines in Fig. 5 show the occupation of the sites by Cu ions for $[Cu^{2+}]_{total} = 10^{-4}$ mol L⁻¹ at pH 5.0. In general, carboxylic sites make a dominant contribution to the Cu binding to HS. For JGFA and JLHA, Cu binding takes place mainly on carboxylic type sites due to their higher abundance of carboxylic type sites with higher affinity, whereas the contribution of phenolic type sites is negligible (small area). This corresponds to the carboxylic-Cu accounting for 95.35% and 96.99% of the amount of Cu bound to JGFA and JLHA, respectively (see Table 3). For the complexation of Cu to PAHA and JGHA, the phenolic distributions are not negligible and, thus, both distributions play a relevant role in the Cu binding. As shown in Table 3, the fraction of carboxylic-Cu is three times more than that of phenolic-Cu for PAHA and JGHA. The Donnan phase is not important in percentage terms (0.03–1.41%) and can be neglected. Therefore, although the CAS depicts only the distribution of chemical interaction involved in the Cu binding to HS, without electrostatic contribution included, it can describe the speciation of Cu bound well for a given total Cu concentration of 10^{-4} mol L⁻¹ at pH 5.0. However, if the Cu

concentration increases, with an increasing percentage of Donnan phase as indicated in Fig. 2, this computation does not fully represent the total binding behavior of Cu to HS.

The average affinity of occupied sites provides rough estimates of the binding strength. A high $\langle \log k'_{Cu} \rangle_{occ}$ suggests that the HS-Cu complexes have a high stability. Table 2 lists the average affinity of occupied sites at given Cu concentration. The values of $\langle \log k'_{Cu} \rangle_{occ}$ are much higher than $\langle \log k'_{Cu} \rangle$ values for corresponding sites, indicating that copper ions preferentially occupy the sites with high affinities. The global $\langle \log k'_{Cu} \rangle_{occ}$ values are responsible for only the chemical binding constants between Cu and HS. The overall binding constants of Cu to HS under given conditions can be assessed by adding the corresponding electrostatic contribution. Due to the small electrostatic contribution under the conditions mentioned above, the $\langle \log k'_{Cu} \rangle_{occ}$ values should be very close to the apparent binding constants. In general, HAs have a higher average binding affinity than JGFA. This is in line with the previous results that most binding constants of HA-like components for Cu binding are higher than that of FA-like fractions [49,50]. The global $\langle \log k'_{Cu} \rangle_{occ}$ ranges from 2.93 to 5.77, which coincides well with experimental values for Cu binding to HS or DOM obtained with various analytical techniques [50–52]. However, the majority of analytical methods permit determination of only global stability constants for Cu binding to HS. It seems to be insufficient to subdivide the binding sites into carboxylic and phenolic groups and to determine their binding constants with Cu.

There is a significant increase of $\langle \log k'_{Cu} \rangle_{occ}$ values for corresponding sites with increasing pH. The values of $\langle \log k'_{Cu} \rangle_{occ}$ for phenolic type sites are larger than that for carboxylic type sites with respect to HAs, roughly indicating that the carboxylic-Cu complex is less stable than phenolic-Cu complex in spite of low Cu occupation

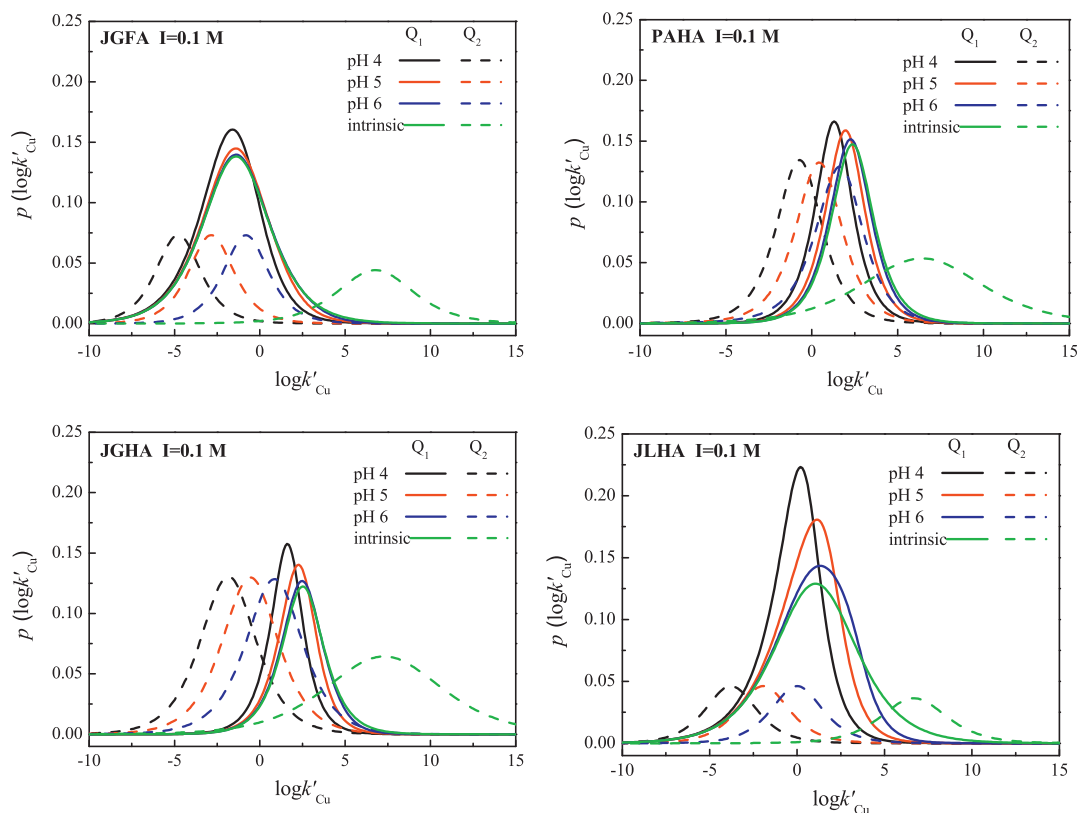


Fig. 4. Carboxylic (solid lines) and phenolic (dashed lines) distributions within the global CAS underlying NICA isotherm at three pH values (4.0, 5.0 and 6.0) and the intrinsic condition ($C_H = 0$), obtained from Eq. (7) with the double logarithmic fitted parameters.

of phenolic type sites. For JGFA, the stability of carboxylic-Cu and phenolic-Cu is comparable. It is apparent that the global $(\log k'_{Cu})_{occ}$ values of PAHA-Cu and JGHA-Cu are larger than that of JLHA-Cu under identical conditions. This result can be attributed to the different coordination environments of Cu. The most possible polydentate configurations chelating Cu^{2+} are adjacent carboxylic or phenolic groups on aromatic rings, such as salicylate-type, catechol-type or phthalate-type [50,53]. We acknowledge that complexes forming five- and six-membered rings are the most stable and the stability constants of the salicylate-Cu and catechol-Cu types are larger than the phthalate-Cu type [15,26]. Combined with speciation calculations, salicylate- and catechol-types appear to be numerous in PAHA-Cu and JGHA-Cu complexes, while the phthalate-type is more probable for Cu binding to JLHA. Although carboxylic group is the predominant site in Cu binding to HS materials, the presence of phenolic type sites contributes to the stability of HS-Cu complexes. The CAS provides assessments of the effective binding strength at given pH, ion strength and Cu concentration. However, the corresponding results need to be further checked with complementary analytical techniques.

5. Conclusions

Binding of Cu(II) to HS of different origins was investigated to study the properties of HS-Cu interaction. Cu binding to HS increased with increasing pH and decreasing ionic strength. The amount of Cu bound to JGFA was smaller than that to HAs due to more carboxylic groups linked to aliphatic carbon backbones compared to HA. To predict the binding properties of Cu to HS, a modeling approach based on the application of the NICA-Donnan model was followed by the binding isotherm experiments. The NICA-Donnan parameter optimization was based on 'low' and 'high' concentration fitting methods to obtain the Cu binding stoichiometry

over a wide range of Cu concentrations. The n_{Cu}/n_H obtained from both fitting modes suggested a significant bidentate coordination. Milne's generic parameters did not provide satisfactory predictions for the present soil HS samples, while material-specific NICA-Donnan model parameters described and predicted Cu binding to the HS well. The low concentration fitted parameters were used to calculate the Cu speciation. Copper binding to HS was mainly by specific interactions. Copper binding to JGFA and JLHA was controlled by carboxylic type sites, while both carboxylic and phenolic type sites played an important role in Cu binding to PAHA and JGHA. This variation largely depended on the difference in the content of functional groups in these samples. To reach a deeper understanding of the specific characteristics of various samples with Cu, CAS associated with NICA isotherm in terms of the effective affinity distributions of each sites at given conditions, distribution of occupied sites etc. was applied. The pH largely modified the location and shape of CAS, i.e., the affinity and heterogeneity of HS sites for Cu. The carboxylic CAS had a stronger conditional affinity than the phenolic distribution for all samples. This was the reason why carboxylic group played an indispensable role in Cu binding to HS. Also, CAS provided a novel and more visualized method to quantify the Cu speciation of specific binding at each affinity. The estimated occupation of HS sites was consistent with the calculation of Cu speciation with NICA-Donnan model at given total Cu concentration. The average affinity of occupied sites $(\log k'_{Cu})_{occ}$ provided estimates of binding strength of carboxylic type sites, phenolic type sites and global behavior. Although carboxylic group was the predominant site in Cu binding to HS materials, the presence of phenolic type sites contributed to form more stable HS-Cu complexes, such as salicylate-type and catechol-type. The calculations merely extrapolated the experiment data of Cu binding to pure HS and the detailed description and understanding in natural systems however still needs further investigations.

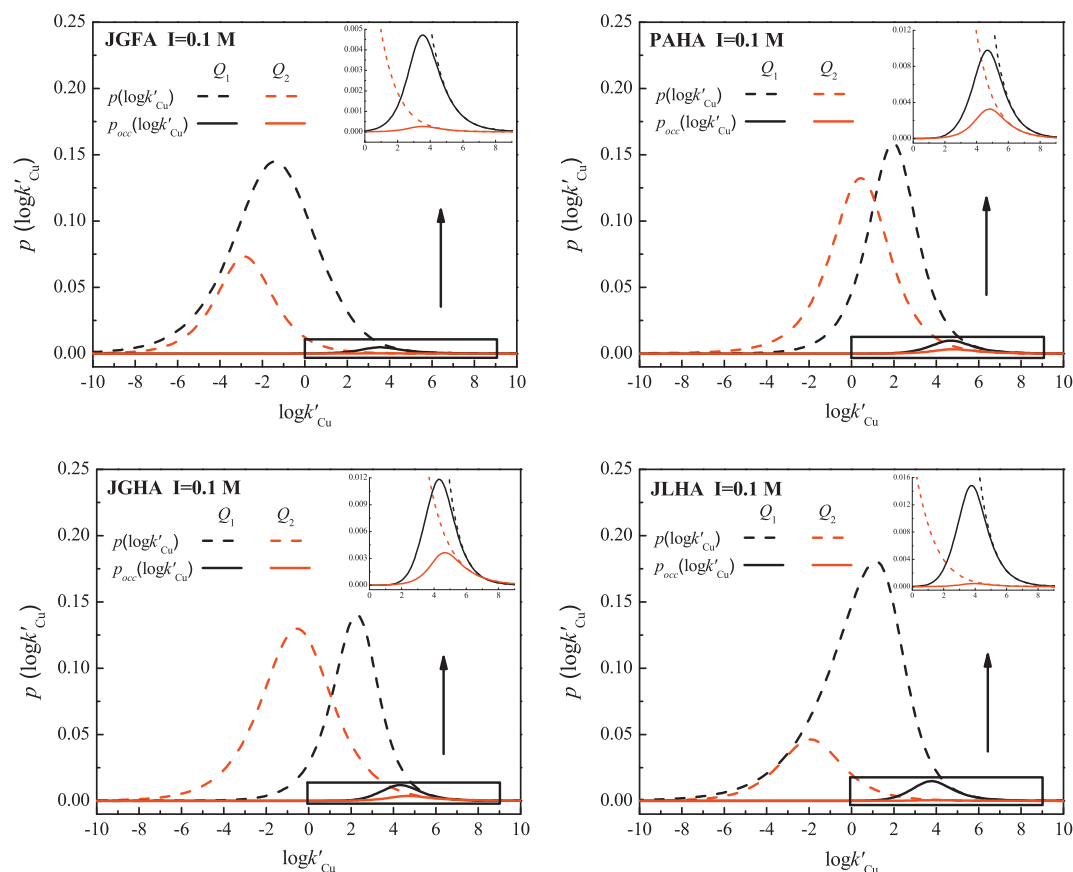


Fig. 5. Occupation of HS sites by Cu at a given condition ($[Cu^{2+}]_{total} = 1 \times 10^{-4} \text{ mol L}^{-1}$, pH 5.0 and $I = 0.1 \text{ mol L}^{-1}$), obtained from Eq. (13). The dashed lines correspond to the CAS of the carboxylic (black) and phenolic (red) sites. The solid lines represent the Cu occupied sites of the corresponding distributions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Average affinity values of CAS associated with NICA isotherm with double logarithmic fitted parameters in Table 1.

	pH	Carboxylic distribution			Phenolic distribution			Bimodal NICA (global)	
		$\langle \log k'_{Cu} \rangle$	σ_{Cu}^2	$\langle \log k'_{Cu} \rangle_{occ}$	$\langle \log k'_{Cu} \rangle$	σ_{Cu}^2	$\langle \log k'_{Cu} \rangle_{occ}$	$\langle \log k'_{Cu} \rangle$	$\langle \log k'_{Cu} \rangle_{occ}$
JGFA	4	-1.90	4.52	3.05	-4.78	3.07	3.04	-2.67	3.05
	5	-1.58	5.22	3.82	-2.85	3.08	3.79	-1.92	3.82
	6	-1.45	5.71	4.76	-0.82	3.09	4.69	-1.28	4.75
PAHA	4	1.27	2.19	4.01	-0.80	3.50	4.19	0.23	4.04
	5	1.90	2.33	4.87	0.30	3.76	5.07	1.10	4.92
	6	2.22	2.50	5.66	1.42	4.18	5.91	1.82	5.77
JGHA	4	1.52	1.77	3.63	-1.87	4.58	4.26	-0.47	3.75
	5	2.12	2.06	4.49	-0.60	4.69	5.12	0.53	4.66
	6	2.40	2.38	5.29	0.78	4.89	5.93	1.45	5.59
JLHA	4	-0.34	3.81	2.92	-3.78	3.68	3.26	-0.98	2.93
	5	0.30	4.93	3.97	-1.93	3.68	4.29	-0.12	3.98
	6	0.69	6.12	5.04	-0.01	3.69	5.34	0.56	5.07

The $\langle \log k'_{Cu} \rangle$ represents the average value of the CAS at given conditions, calculated with Eq. (10). σ_{Cu}^2 is the variance of CAS calculated with Eq. (11). $\langle \log k'_{Cu} \rangle_{occ}$ is the average affinity of occupied sites calculated using Eq. (14).

Table 3

Copper bound speciation onto HS at pH 5.0 in 0.1 M KNO_3 for $[Cu^{2+}]_{total} = 10^{-4} \text{ mol L}^{-1}$.

	JGFA	PAHA	JGHA	JLHA
Carboxylic-Cu (%)	95.35	78.99	76.52	96.99
Phenolic-Cu (%)	3.23	21.98	23.45	2.88
Donnan (%)	1.41	0.03	0.03	0.13

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcis.2016.03.066>.

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