Copper complexation by dissolved organic matter and uncertainty assessment of their stability constants

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Abstract

The interaction of Cu with dissolved organic matter (DOM, extracted from an organic forest floor) was investigated and the resulting data was evaluated in terms of their uncertainty. The speciation of Cu over ‘free’ Cu (as analysed by diffusive gradients in thin films (DGT)), dissolved Cu–DOM complexes and precipitated Cu–DOM was determined as a function of pH (3.5, 4.0 and 4.5) and Cu/C ratio. The dissolved organically bound fraction was highest at pH 4.5, but this fraction decreased with increasing Cu/C ratio, which was observed for all pH levels. In the range of Cu/C = 7 × 10⁻⁵–2.3 × 10⁻² (mol/mol) the precipitated fraction was very small. The speciation of both Al and Fe was not affected by increasing Cu concentrations. From a continuous distribution model using the Scatchard approach, we calculated the optimal fit and corresponding upper and lower 95% uncertainty bounds of the overall stability constants (Kₒ) with the shuffled complex evolution Metropolis (SCEM) algorithm. Although the optimal equation fitted the data very well, the uncertainty of the, according to literature, most reliable approach to establish stability constants, was still large. Accordingly, the usually reported intrinsic stability constants exhibited large uncertainty ranging from log Kᵢ = 6.0–7.1 (optimal 6.7) for pH 3.5, log Kᵢ = 6.5–7.1 (optimal 6.8) for pH 4.0, and log Kᵢ = 6.4–7.2 (optimal 6.8) for pH 4.5 and showed only little effect of pH.

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1. Introduction

One of the major issues in soil and environmental sciences is the occurrence and fate of heavy metals in soils. Depending on the concentration, heavy metals (e.g. Cu, Cd, Pb) can be either essential nutrients or toxic to biota. Excessive use of fertilizers, industrial activity and the dump of waste materials have caused undesirably high concentrations of such metals in many soils. An additional environmental concern lies in the change of agricultural land into grass and forest areas to reduce the agricultural overproduction. In The Nether-
strongest complexes with DOM (Schnitzer, 1978; Evans, 1989; Stevenson, 1994; Pandey et al., 2000). Therefore, in contrast to e.g. Cd, which preferably forms soluble complexes with Cl, the mobility of Cu will be mainly influenced by its interaction with DOM (De Wit, 1992; Temminghoff et al., 1995, 1997). Moreover, Cu binding to DOM is competed by Al and Fe that form stronger complexes with DOM and are present in considerable amounts in most acidic forest soil solutions (Strobel et al., 2001a; Titeux et al., 2002).

The bioavailable fraction of metals is normally considered as the part that is ‘free’, i.e. not complexed with any organic species in solution or adsorbed to solid soil particles (Zhang et al., 2001). Recently, the method of diffusive gradients in thin films (DGT) has been developed to distinguish (hydrated) metal cations and small labile dissolved complexes from metal–DOM complexes to measure free metals (Zhang and Davison, 1995; Zhang et al., 1998). Apart from the aforementioned heavy metals also free Al and Fe can be successfully determined in a single measurement with this technique (Jansen et al., 2001, 2002).

The maximum binding capacity (MBC) of a given organic substrate to complex metals depends on the total number of (strong and weak) binding sites, which is related to the number of functional groups. The total acidity, i.e. the number of carboxylic acids and phenolic hydroxyl groups, is often used as a measure for the binding capacity (Gerke, 1994; Stevenson, 1994). However, the total acidity is not the most accurate measure of metal binding capacity, because organic matter has other binding sites as well (Evans, 1989; Stevenson, 1994). Another way to assess the MBC is by the Langmuir adsorption equation, either a one- or two-surface adsorption approach (Stevenson, 1994; Logan et al., 1997).

A second parameter that determines the binding of a given metal to an organic ligand, and consequently its behaviour in (soil) solution, is the binding strength, generally expressed as the (overall) stability constant ($K_o$). Many models have been developed to determine these $K_o$ values, but most of them consider complexes with only one, two or three types of binding sites (e.g. Ruzic, 1982; Buffle, 1990; Stevenson, 1994; Luster et al., 1996; Town and Filella, 2000). These different approaches have resulted in a large variety of reported binding constants and contradictive interpretations (Stevenson et al., 1993). An obvious reason for these variations is the use of different organic ligands and other divergent experimental conditions (Town and Filella, 2000). Another major cause for the observed differences, for example when the two-component Scatchard approach is used, is the somewhat arbitrary fitting of the two straight lines (Stevenson et al., 1993). As a consequence, the resulting $K_o$ values are subject to large errors (Logan et al., 1997). Therefore, a continuous distribution model seems not only the most promising but also the most realistic approach (Stevenson et al., 1993; Stevenson, 1994).

The binding of Cu to organic matter, humic substances or their fractions (humic acid, fulvic acid) has been studied extensively (e.g. Buffle, 1990; Stevenson et al., 1993; Stevenson, 1994; Logan et al., 1997; Pandey et al., 2000; Town and Filella, 2000; Titeux et al., 2002). However, data of Cu binding to soil derived DOM, the most mobile and thus potentially the most active transporters of metals in soil are scarce (Evangelou and Marsi, 2001; Strobel et al., 2001b) in contrast to DOM from natural waters (Buffle, 1990; Town and Filella, 2000).

In this paper we aim to describe the behaviour of Cu in the presence of DOM derived from the H horizon of an acidic sandy forest soil covering a wide range of Cu concentrations (Cu/C molar ratio $7 \times 10^{-5}$–$2.3 \times 10^{-2}$). The speciation of Cu over the free metal fraction as measured by DGT, the dissolved (organically) complexed fraction and the precipitated fraction was determined. Additionally, the competitive effect of increasing Cu concentrations on the speciation of the initially present Al and Fe was examined. Furthermore, an attempt was made to calculate the binding constants of Cu to DOM during this titration using a continuous distribution model. To assess the optimal parameters of the distribution model as well as their uncertainty we applied the recently developed shuffled complex evolution Metropolis (SCEM) (Vrugt et al., in press). From this analysis we computed predictive equations from which not only the MBC and $K_o$ values were derived, but also their uncertainty. Additionally, to compare our results with data reported in literature and to show their sensitivity to uncertainty, we calculated intrinsic stability constants ($K_i$).

2. Materials and methods

2.1. DOM preparation

DOM was extracted from the H horizon (pH (H$_2$O) 3.8) of a Fimic Anthrosol (FAO, 1988) located at the Buunderkamp (The Netherlands). Field-moist material was sieved (2 mm) and subsequently shaken overnight with nano-pure water in a ratio of 1:3 (w/w). After centrifugation (30 min at 16000 g) the solution was filtrated over 0.45 μm (ME membrane filters; Schleicher and Schuell). NaN$_3$ was added resulting in a 0.01 M concentration to prevent microbial degradation (De Maagd et al., 1998). In addition, it ensured the solutions to have a relatively constant ionic strength during the (initial) titration steps as well as a minimum ionic strength needed for DGT (Alfaro-De la Torre et al., 2000). The solution was divided into three batches, which were adjusted to pH 3.5, pH 4.0 and pH 4.5, respectively, using 1 M HNO$_3$ or 1 M KOH. These pH
values are representative for the range observed in soil solutions of this acidic sandy soil (Tietema et al., 1993).

2.2. Titration with Cu

Stock solutions of Cu(NO₃)₂ were prepared in such a way that at every titration step no more than 2 ml of a given stock solution had to be added to the DOM solutions. This resulted in four stock solutions with concentrations of 1.94, 12.1, 60.6 and 758 mmol l⁻¹ Cu, respectively. Cu was titrated to the DOM solutions by adding a stock solution to each of the aforementioned DOM solutions of initially 1 l, which were shaken for 24 h to reach equilibrium at a constant temperature of 20 °C. After rigorous shaking of the glass container to achieve a homogeneous suspension about 35 ml was collected for further analysis. This was followed by a next addition of a stock solution to the reaction container, after which the total solution was adjusted to the desired pH by adding small quantities of 1 M KOH and weighed in order to calculate the theoretical concentrations and the molar Cu/C ratios.

The collected suspensions were centrifuged and filtered. The finally obtained supernatants were analysed for C as DOC by colorimetric determination on a Skalar continuous flow autoanalyzer, and for metals by a Perkin Elmer Optima 3000XL inductively coupled plasma–continuous flow autoanalyzer, and for metals by a Perkin Elmer Optima 3000XL inductively coupled plasma–optical emission spectrometer (ICP–OES).

Each experiment was performed in triplicate for each pH level. Before use, all glassware was rinsed with acid and nano-pure water to avoid metal contamination.

2.3. Diffusive gradients in thin films

Standard DGT units for metals were obtained from DGT Research Ltd, UK, and assembled according to the procedures described elsewhere (Zhang and Davison, 1995). The units contained a Chelex 100 chelating resin, after which the total solution was adjusted to the desired pH by adding small quantities of 1 M KOH and weighed in order to calculate the theoretical concentrations and the molar Cu/C ratios.

The collected suspensions were centrifuged and filtered. The finally obtained supernatants were analysed for C as DOC by colorimetric determination on a Skalar continuous flow autoanalyzer, and for metals by a Perkin Elmer Optima 3000XL inductively coupled plasma–optical emission spectrometer (ICP–OES).

Each experiment was performed in triplicate for each pH level. Before use, all glassware was rinsed with acid and nano-pure water to avoid metal contamination.

2.4. Calculation of free metal, metal–DOM complexes and precipitates

The free metal mass from the DGT analyses was calculated from the amount of metal determined by ICP–OES in the elution samples of the chelating resin, using the DGT formulas (Zhang and Davison, 1995):

\[ m = \frac{C_e(V_{\text{HNO}_3} + V_{\text{gel}})}{f_e} \]

\[ M = \frac{m \Delta g}{D t A} \]

where \( m \) denotes the metal mass (µmol) captured on the chelating resin and is calculated from the metal concentration in the elution sample \( (C_e, \mu\text{mol} l^{-1}) \) the volume of the elution fluid \( (V_{\text{HNO}_3} = 1.0 \times 10^{-3} \text{ l}) \) and the chelating resin \( (V_{\text{gel}} = 1.6 \times 10^{-3} \text{ l}) \), and an elution factor \( (f_e = 0.8) \) needed to compensate for incomplete metal removal from the resin (Zhang and Davison, 1995).

From \( m \), the free metal concentration \( M \) (µmol cm⁻³) is calculated by taking into account the thickness of the diffusion gel layer \( (\Delta g = 0.094 \text{ cm}) \), the diffusion coefficient of the metals in the hydrogel \( (D, \text{cm² s}^{-1}) \), the deployment time \( (t = 5.76 \times 10^4 \text{ s}) \) and the area of exposure \( (A = 3.14 \text{ cm}^2) \). The diffusion coefficients in the APA hydrogel are similar to those in water, but vary slightly depending on the cross-linker used during the manufacturing of the hydrogel (Li and Gregory, 1974; Zhang and Davison, 1999). Accurate diffusion coefficients were supplied by the manufacturer. For the metals of interest at 20 °C these are: Cu: 5.42 \times 10^{-6} \text{ cm² s}^{-1}; Al: 4.14 \times 10^{-6} \text{ cm² s}^{-1} and Fe: 5.32 \times 10^{-6} \text{ cm² s}^{-1}.

We defined precipitation of metals and carbon as the initial plus added amounts of a giving element (theoretical amounts) minus the dissolved amounts after centrifugation and filtration. From the dissolved amounts the free metal concentration was subtracted to obtain the extent of dissolved metal–DOM complexation.

2.5. Calculation of the stability constants and binding capacity

A number of models have been developed to determine the stability constants of complexation of metals to organic matter. The most promising and frequently applied of these are the models based on the Scatchard approach (Buffle, 1990; Stevenson and Chen, 1991; Stevenson et al., 1993; Gerke, 1994; Logan et al., 1997), which is derived as follows. The reaction between metal \( (M) \) and organic ligand \( (L) \) with successive reaction products \( (LM, LM_2 \text{ to } LM_n) \) can be described as

\[ K_1 = \frac{[LM]}{[L] + [M]}; \quad K_2 = \frac{[LM_2]}{[LM] + [M]}; \quad K_n = \frac{[LM_n]}{[LM_{n-1}] + [M]} \]
The extent of binding is expressed as

\[ v = \frac{M_b}{L_i} = \frac{[LM] + 2[LM_2] + \cdots + n[LM_n]}{[L] + [LM] + \cdots + [LM_n]} \]

(4)

where \( M_b \) is the concentration of metal bound to DOM, and \( L_i \) the total concentration of the ligand, i.e. the total number of binding sites. Writing this Eq. (2) in terms of stability constants yields:

\[ v = \frac{K_i[M] + 2K_iK_2[M]^2 + \cdots + nK_iK_2\cdots K_n[M]^n}{1 + K_1[M] + K_1K_2[M]^2 + \cdots + K_1K_2\cdots K_n[M]^n} \]

(5)

For independent and identical binding sites this equation can be written as

\[ v = \frac{nK_o[M]}{1 + K_o[M]} \]

(6)

where \( K_o \) is the overall stability constant. Eq. (6) can be rearranged to give:

\[ \frac{v}{M} = nK_o - vK_o \]

(7)

which is the Scatchard equation. Plotting \( v/M \) against \( v \) yields \( K_o \) as the slope.

To apply the Scatchard approach, the extent of binding (\( v \)) has to be calculated from the total number of binding sites. From the Langmuir adsorption equation

\[ x = \frac{(MBC)K_i[M]}{1 + K_i[M]} \]

(8)

the so-called MBC can be derived (Stevenson, 1994; Logan et al., 1997). The \( x \) represents the concentration of metal bound to 1 mole of DOM, and \( K_i \) a constant that stands for the affinity between a metal ion and DOM.

Usually, the Scatchard plot is divided into two or three segments to give an equal number of slopes and stability constants (Stevenson et al., 1993; Logan et al., 1997). However, there is no ambiguously correct way to divide the plot into two or three segments (Stevenson et al., 1993). Consequently, this approach is susceptible to a degree of subjectivity. To avoid this potential problem, we fitted a non-linear regression line through the data set (\( v, v/M \)).

As the estimation of the parameters of the Scatchard equation involves a variety of possible errors, like analytical and model errors, an uncertainty analysis of the finally optimised curve constitutes an important part of this estimation. Therefore, we applied the SCEM algorithm, a general-purpose method suited for optimisation and uncertainty assessment of model parameters (Vrugt et al., in press). The algorithm is a modified version of the original SCE global optimisation algorithm developed by Duan et al. (1993). It operates by selecting and modifying an initial population of parameter sets merging the strengths of the Metropolis algorithm (Metropolis et al., 1953), with the concepts of controlled random search (Price, 1987), competitive evolution (Holland, 1975) and complex shuffling (Duan et al., 1993) in order to evolve the population to a stationary posterior target distribution. The SCEM algorithm is different from the original SCE algorithm presented by Duan et al. (1993) in two important ways. Both these modifications are necessary to prevent the search from becoming mired in a small basin of attraction and thus to arrive at a stationary posterior target distribution. First, the downhill Simplex method in the SCE-UA algorithm is replaced by a Metropolis-annealing covariance based offspring approach, thereby avoiding a deterministic drift towards a single best parameter set. Second, the SCEM-UA algorithm does not further subdivide the complex into subcomplexes during the generation of the offspring (parameter sets) and uses a trapezoidal weight distribution to ensure that the chance that better parents (parameter sets) are replaced in the complex is higher than that of worse parents. This latter modification ensures a correct estimation of the uncertainty associated with the final parameter estimate as it counts any tendency of the search to terminate occupations in the less-productive part of the parameter space.

The intrinsic stability constants (\( K_i \)) were derived from (linear) extrapolation of the first six data points to \( v = 0 \) in the plot of \( \log K_o \) against \( v \), after which the intercept with the \( y \)-axis yielded \( K_i \). As stated earlier, they were only calculated to compare them with those presented in the literature and to show the susceptibility of \( K_i \) to large uncertainty during data analysis.

3. Results and discussion

3.1. Dissolved organic matter

The initial composition of the DOM solution is given in Table 1. The DOM contained trace amounts of Cu, while relatively high concentrations of Ca, Mg, Al and Fe were present. The trivalent metals Al and Fe and the divalent Cu were predominantly present as metal–DOM complexes, indicative of their relative strong binding to DOM (Evans, 1989; Stevenson, 1994). For the other metals no speciation is given, because we experienced that DGT has problems in measuring weakly binding cations at low pH values. In the range in which Cu was added, precipitation of DOM was only a minor process. Only after the largest addition, giving a Cu/C ratio of 0.15, DOM precipitation was significant (Fig. 1). It is clear that the highest degree of precipitation was found at the highest pH, where more deprotonated binding sites were present giving DOM a higher affinity to bind metals. This resulted in a higher amount of binding sites...
occupied by Cu producing more charge-compensated DOM-Cu complexes and thus a higher degree of precipitation. By contrast, Temminghoff et al. (1998) found only a minor pH effect, between pH 4 and pH 6, on coagulation of a humic acid upon Cu addition.

### 3.2. Cu speciation

Fig. 2 shows the speciation of Cu over the free fraction, the dissolved complexed fraction, and the precipitated fraction at pH 3.5, pH 4.0 and pH 4.5 in a range Cu/C molar ratio of \(7 \times 10^{-5} - 2.3 \times 10^{-2}\). At all three pH levels the fraction that precipitated was lowest. At low Cu/C ratios, the majority of Cu was bound to DOM in solution, but this fraction decreased with increasing addition of Cu. At low pH, the point where the free fraction exceeded that of the complexed fraction in solution takes place at a lower Cu/C ratio than at high pH. For pH 3.5 this point of intersection lies approximately at Cu/C = 0.0027, for pH 4.0 it is at Cu/C = 0.0045, and for pH 4.5 this point is at around Cu/C = 0.018. This behaviour, a higher degree of dissolved metal-DOM complexes with increasing pH, is explained by the fact that at high pH more functional groups at the DOM molecules are deprotonated, resulting in a lower

<table>
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<th>Species</th>
<th>Free fraction (mol/mol)</th>
<th>pH 3.5</th>
<th>pH 4.0</th>
<th>pH 4.5</th>
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<td>C(^a)</td>
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<td></td>
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<tr>
<td>Al</td>
<td>22.1</td>
<td>0.22</td>
<td>0.17</td>
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<tr>
<td>Fe(II)(^b)</td>
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<td>0.21</td>
<td>0.13</td>
<td>0.05</td>
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<tr>
<td>Fe(III)(^b)</td>
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<td>0.13</td>
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<tr>
<td>Mg</td>
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<td></td>
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<tr>
<td>Ca</td>
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</tr>
<tr>
<td>Mn</td>
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<tr>
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<tr>
<td>S(^d)</td>
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<tr>
<td>Cl</td>
<td>489</td>
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<td></td>
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</tr>
</tbody>
</table>

\(^a\)DOC.  
\(^b\)Fe(II)/Fe(III) speciation not measurable after DGT.  
\(^c\)ortho-P + DOP.  
\(^d\)SO\(_4\) + DOS.
proton competition and higher affinity of DOM to bind metals. Other organic ligands (humic acid and DOM from corn tissue) displayed a similar trend (Temminghoff et al., 1994; Evangelou and Marsi, 2001). Such tendency was also observed for Al, Fe(II) and Fe(III) using the same DOM solutions (Jansen et al., 2002). However, the latter comparison is only valid at relatively low M/C ratios, because Al and Fe precipitate in significant amounts after a certain M/C ratio (Jansen et al., 2002; Nierop et al., in press), while Cu hardly induced precipitation, except for very high Cu/C ratios. The reason of the low precipitation potential of Cu may lie in the fact that Cu binds predominantly in a 2:1 stoichiometric arrangement (Logan et al., 1997) or at least partly (Stevenson et al., 1993) with most likely Cu as a bridge between two different DOM molecules rather than within one and the same molecule. This would result not only in relatively stable metal–DOM complexes, but also in an incomplete compensation of charge thereby inhibiting precipitation. The constant and low extent of precipitation along the titrations before the last addition allowed us to calculate the binding of Cu to DOM in solution.

Fig. 3. Distribution of free Al (○), dissolved Al–DOM complexes (▲) and precipitated Al (◇) at pH 3.5 (a), at pH 4.0 (b) and at pH 4.5 (c) as a function of the molar Cu/C ratio.

Fig. 4. Distribution of free Fe (○), dissolved Fe–DOM complexes (▲) and precipitated Fe (◇) at pH 3.5 (a), at pH 4.0 (b) and at pH 4.5 (c) as a function of the molar Cu/C ratio.
3.3. Competition of Al and Fe

Figs. 3 and 4 display the speciation of Al and Fe over free, dissolved organically complexed and precipitated fractions as affected by pH and Cu concentration, as expressed by the Cu/C ratio. For Al, the differences in fraction distributions showed hardly any pH effect. Compared to Cu, a relatively larger part of Al precipitated, while the majority (60–70%) was present as dissolved Al-DOM complexes. Initially, the Cu/Al ratio was about 0.01, but increased up to 3 during the titration experiment. Notwithstanding the large Cu addition, the data clearly showed that Cu was not capable of replacing Al from either precipitated or dissolved complexes. This demonstrates the much stronger binding strength of Al to DOM compared to Cu.

For Fe, also the majority (up to 75%) was present as dissolved Fe-DOM complexes. The free fraction was significantly greater than the precipitated fraction at pH 3.5, but with increasing pH the latter increased and even (slightly) exceeded the free Fe fraction. Similar to Al, the distribution was not influenced by Cu addition, while the Cu/Fe ratio increased from 0.02 to 7. Because Fe binds more firmly to DOM than Al, even a seven times higher amount of Cu did not affect the interaction between Fe and DOM. Another explanation of the incapability of Cu to affect Al and Fe complexation to DOM may be the different preferences of the mentioned metals for certain functional groups (Evans, 1989; Stevenson, 1994). As aforementioned, Fe and Al are considered to be hard acids that bind to hard bases, while Cu is a softer acid and binds preferably with a softer base (Evans, 1989). Furthermore, Al and Fe are likely to be bound to the strongest binding sites for these metals, making it even more difficult to be replaced by any competing cation. On its turn, Cu has been found to experience competition from other cations, like Ca, upon complexation with DOM (Temminghoff et al., 1998).

3.4. Stability constants of Cu to DOM in solution

The MBC values of the DOM as calculated by the Langmuir approach are presented in Table 2. Additionally, the 95% confidence intervals, being associated with the most optimal MBC values, derived using the SCEM algorithm, are also included. From the MBC values it is apparent that if the proton activity is 10 times lower (pH 4.5 vs. pH 3.5), the (optimal) MBC increases only by a factor of 2. This observation is consistent with data calculated by Logan et al. (1997) for Cu and humic acid/peat binding in the range of pH 4.8–5.8 and pH 4.0–5.0, respectively. However, the MBC value of the DOM at pH 4.5 is about six times higher than that of peat derived humic acid at pH 4.8, and even higher than that of the peat itself (Logan et al., 1997). Its greater hydrophilicity implies that DOM will have more functional groups than both the humic acid and peat, and is therefore able to bind more metals, which could explain this discrepancy.

The resulting Scatchard plots are presented in Fig. 5. The most appropriate transition model to fit the experimental data under consideration appeared to be the non-linear equation

$$\frac{v}{M} = av^{-b}$$

from which stability constants could be derived by differentiation:

$$K_a = -\frac{d(v/M)}{dv} = abv^{(-b-1)}$$
Log \( K_0 \) as a function of the extent of binding (\( \nu \)) is graphically depicted in Fig. 6. Using the optimal fit, equal \( \nu \) values resulted into highest stability constants at pH 3.5. However, if equal amounts of Cu are bound to DOM at varying pH, thus with lowest \( \nu \) at pH 4.5, the stability constants \( K_0 \) are now highest at pH 4.5. DOM has a MBC of about two times higher than that of DOM at pH 3.5. This means that twice as much of Cu can be complexed by DOM at pH 4.5, which sheds a different light on the obtained \( K_0 \) values. As mentioned earlier, Fig. 2 shows indeed that more Cu is bound to DOM at pH 4.5 than at pH 3.5 at a given Cu/C ratio.

The relatively high \( R^2 \) values demonstrated the validity of the non-linear regression model to fit the measured data. However, the uncertainty associated with log \( K_0 \) values close \( \nu = 0 \) is large. As a result, a broad range of intrinsic \( K_i \) values is obtained after extrapolating the regression line \( \nu = 0 \) (Table 2), which includes most of the reported ones in the same pH range (e.g. Buffle, 1990; Stevenson and Chen, 1991; Logan et al., 1997; Pandey et al., 2000; Town and Filella, 2000; Evangelou
and Marsi, 2001). One obvious reason may be the different analytical techniques to measure Cu and their implicit errors, and the varying concentration ranges of Cu used. Moreover, these values are based on humic and fulvic acid fractions, and the closest resemblance of the log $K_i$ values we determined were found with those reported for DOM, i.e. 6.93–7.25 at pH 4.0 (Evangelou and Marsi, 2001), 5.64–5.89 at $\approx$ pH 5 under oak (Strobel et al., 2001b) and 7.47 for the strongest binding sites of a leaf litter extract at pH 6 (Luster et al., 1996).

However, as aforementioned in the Materials and Methods section, an important reason of the differences lies in the way these $K_i$ values are obtained. In fact, the equation to fit our data best was a non-linear hyperbolic function that does not include an intercept with the y-axis so that extrapolation of $v = 0$ was impossible. Only when a linear function was applied to data close to the y-axis, as used in the ‘two or three segment approach’, it was feasible to ’produce’ such $K_i$ values. Thus, only when arbitrary choices were made, $K_i$ values could be calculated, and with a large degree of uncertainty. The question arises whether it is still appropriate to report such intrinsic stability constants, and if it would not be better to use $K_o$ data in the form of a function of. Even then much uncertainty is produced, which is likely caused by the errors during the determination of free metals. If one wants to calculate stability constants, one should be very cautious about their values, both the figure itself as the implications for the environmental fate of the metals.

4. Conclusions

The presented data showed that the extent of Cu binding to DOM as affected by pH depends on the number of binding sites (MBC) rather than on the binding strength ($K_o$, $K_i$). Acidification of soil in case of the afforestation of agricultural land would result in a higher degree of free Cu and thus also in a higher bioavailability or toxicity. Conversely, the mobility of Cu would decrease as the pH decreases.

Cu did not affect the binding of Al and Fe to DOM, irrespective of its concentration. As Al and Fe are strong precipitators of DOM (Nierop et al., in press), this may have consequences for Cu as well. A lower DOM concentration will leave more free Cu and thus the mobility of Cu decreases, whereas its bioavailability increases.

Finally, the calculation of overall stability constants $K_o$, especially those of the intrinsic stability constants $K_i$, is subject to a large degree of uncertainty even though the data as expressed in Scatchard plots could be fitted well. Along with the different organic ligands used to study Cu binding to organic matter (fractions), the large uncertainty in the obtained $K_i$ values may explain the conflicting information that exists in literature (Stevenson and Chen, 1991; Stevenson et al., 1993). Not understanding the fact that analytical techniques to measure metals affects the quality of the data set, our optimisation of the equation to fit the Scatchard plot and the derived stability constants using SCEM can result in more consistent values in the form of $K_o$ as a function of rather than estimating arbitrary $K_i$ values not only for Cu–DOM complexation particularly, but also for other metal–(D)OM interactions. In particular, the use of DGT to determine free metals in combination with SCEM is a promising approach to quantify the speciation and complexation of Al and Fe by DOM, which we are currently doing.

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References


