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Review

Metal speciation in natural waters with emphasis on reduced sulfur groups as strong metal binding sites[☆]

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Abstract

The proper application of biotic ligand models to predict metal toxicity depends on accurate prediction of metal binding to sites on natural organic matter (NOM) which compete with the 'biotic' ligand for available metal. A hard and soft metal classification along with associated ligand groups (carboxyl, phenolic, amino, sulfidic) are used as a basis to predict metal speciation in the presence of aqueous organic matter. Compilation of conditional metal formation constants ($\log K'$) are made for each ligand type using model ligands. Model ligands were chosen to reflect those found in NOM and bio-organic media. Total ligand concentration (L_T) estimates for different natural settings and $\log K'$ values are then used to generate a L_T - $\log K'$ distributions for a specific metal. A plot for Cu(II) gives a similar trend as a compilation of measured data for natural environments. A $\log K'$ - L_T plot for Ag(I) shows a much more discrete binding pattern than for Cu(II). Estimation of speciation of a specific metal in a specific environmental setting and to design speciation and toxicological experiments requires accurate knowledge of the functional groups in NOM.

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Keywords: Copper; Silver; Natural organic matter; Metal speciation; Functional groups; Sulfide; Biotic ligand model**1. Introduction**

The framework for any biotic ligand model (BLM) is essentially a chemical equilibrium model for metal speciation in aqueous systems (Meyer et al., 1999; De Schampheleere and Jansen, 2002; Crist et al., 2002; McGeer et al., 2000). Chemical equilibrium models applicable to natural systems include RANDOM (Murray and Linder, 1983; Bryan et al., 1997; Woolard and Linder, 1999), MINTEQA2 (Allison et al., 1991), PHREEQE (Parkhurst et al.,

1980) and WHAM (Tipping and Hurley, 1992; Tipping, 1998). The solution to all chemical equilibrium problems depends on simultaneously satisfying all the mass balance and mass action equations for the defined system. For metal speciation in aqueous systems this includes inorganic complexation to ligands such as hydroxide or fluoride and mineral surfaces as well as organic complexation to binding sites within natural organic matter (NOM) or on biotic surfaces. BLMs ideally should include all these ligands and also a 'biotic' ligand that competes with the existing ligands for available metal; whatever the metal of interest might be. The focus of this current work is to summarize metal binding to sites within NOM that compete for metal binding at biotic ligands. Silver and copper are presented as exam-

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ples emphasizing the importance of reduced sulfur as strong metal binding sites in NOM.

The parameters used as input into chemical equilibrium and BLMs are the number and concentration of ligands (including the biotic ligand) and corresponding metal association reactions and stoichiometries for each metal–ligand species as well as the associated stability constants (K) for each reaction. These parameters can be summarized as a total concentration (L_T) for each ligand and a conditional stability constant K' for the one to one association reaction of the metal with the ligand. In general only one to one stoichiometry reactions are considered (see below) and the formation constant is designated conditional because the metal–ligand association reaction is dependent on the conditions in solution; in particular, pH and ionic strength. In particular, pH has a strong control on metal speciation because it determines the degree of metal hydrolysis, polymerization, aggregation, precipitation and protons compete for available metal binding sites on ligands.

For Ag, there has been some recent work in defining the speciation parameters ($\log K'$ and L_T) for the biotic ligands (McGeer et al., 2000) but there is very little information available for silver speciation parameters with NOM. The reason for there being relatively few studies (Adams and Kramer, 1999b; Smith et al., submitted for publication) on silver speciation with NOM is that silver occurs at very low levels (nM) and analytical methods for speciation at this level are quite difficult. It is possible to get an idea of silver speciation in natural aqueous systems even without analytical data by studying model ligands that represent possible binding sites in NOM.

This paper summarizes chemical speciation in natural waters and for cellular functional groups with regard to potentially toxic metals and protons. Results are generalized from model compounds. First a classification for metals is given. Secondly, the kind of and abundance of ligands in natural waters and for functional groups in enzymes is stated. Binding constants ($\log K'$) and site capacities (L_T) are then compiled for each significant functional group. A generalized summary for each metal can then be developed by statistical integration of these data. The resulting information can be used along with ligand and metal concentration data to postulate predominant species as well as a guide for the examination of existing literature on speciation and design of experiments. This

approach is similar to RANDOM2 (Woolard and Linder, 1999) except here we use statistical integration of model compound data as opposed to actually simulating organic macromolecules from model compound building blocks.

Tipping and Hurley (1992) developed a metal speciation model (WHAM) based upon a slightly different but comparable approach. Their model considered carboxyl groups as the predominant ligand, and later amino groups, modeled by ammonia binding, were added for stronger ligand sites (Tipping, 1998). An electrostatic term was included, and adjustments were made to account for multi-carboxyl group and mixed group ligand binding to a given metal.

Recent observations on the common occurrence of reduced sulfide species at the nano-molar level in fresh and marine environments (Bowles et al., submitted for publication), and especially at outfalls of waste water treatment plants (WWTPs), emphasizes the importance of these strong binding ligands for metals, such as silver, mercury, copper, lead and zinc.

The approach presented here is to survey the literature for functional groups important for metal binding both to organic and biotic material. We chose copper and silver as two different examples, since silver binds strongly to reduced sulfur species, and copper can bind well to many functional groups. In addition, comparisons can be made between the predicted binding of copper to functional groups with the relatively abundant experimental data for copper.

2. Metal–ligand associations

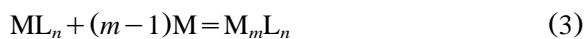
The general equation for formation of a metal (M)–ligand (L) species is:



where m and n are the number of moles of metal, M, and ligand, L, respectively. Eq. (1) is given for a general binary polynuclear species. More often, mononuclear species are assumed:



The relationship between mononuclear and polynuclear species can be seen by combining Eqs. (1) and (2) to give:

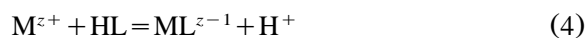


Thus, the concentration of M, $[M]^{(m-1)}$, would

define whether ML_n or M_mL_n would be the predominant species. The ‘mononuclear wall’ has been proposed for the concentration of total metal when one percent of the total species is polynuclear (Stumm and Morgan, 1996). Thus, the ‘analytical window’ (Altmann and Buffle, 1988) under which speciation is assessed is crucial to the nature of the specific species for a defined metal and ligand system and its relationship to a toxicological effect as well as its significance in defining a natural environment in various BLM programs. Only mononuclear species are considered in most BLM programs; e.g. Di Toro et al. (2001), although strongly bound species are possibly polynuclear (Bell and Kramer, 1999).

The term ‘ligand’ is defined here as anions, molecules, clusters, polymers and small particulates to which a metal cation binds. The nature of binding can range from mostly electrostatic to mainly covalent. Metal–ligand complexes range from simple inorganic species such as $CuOH^+$ or $CuCO_3^0$ to complicated organic macromolecules with metal binding functional groups such as carboxyl ($M-OOCR$), amino ($[M-NH_2R]^+$, $M-NHR$), phenolic ($M-OAr$), metal sulfides ($M-SM'$), and thiolate–sulfide ($M-SR$) as found in NOM or in enzyme ligands. Typical molecular weights are from a few 100 to 10 000 Da or more. The definition of ‘soluble’ and ‘filtered’ and operational biases of separation impact these terms and values.

Functional groups (L) may be protonated and compete with metal binding:

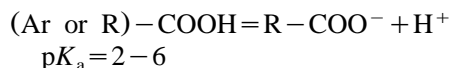


and the proton effect on the binding constant for a simple monoprotic ML ($z=1$) is:

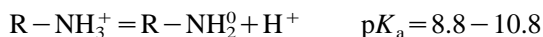
$$K' = \frac{K'_{ML}}{K'_{HL}} \quad (5)$$

where $K'_{ML} = [ML]/[M^+][L]$ and $K'_{HL} = [HL]/[H^+][L]$.

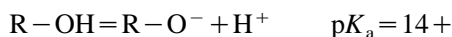
NOM and bio-organic matter have similar functional groups (Cox et al., 1999). Approximate acid pK_s of specific functional groups are similar (Laidler and Bunting, 1973). These active groups are typically attached to aromatic rings, at the end of aliphatic chains, on oxidatively degraded carbohydrates, or degraded peptides. Thus for aliphatic carboxylic acids, such as acetic or formic or aromatic acids, such as benzoic,



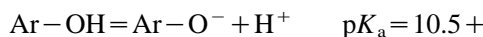
and amino groups, such as on lysine,



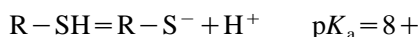
and for alcohols (e.g. as in threonine or serine),



and for phenolics (e.g. as in tyrosine),



and for thiol groups, such as in cysteine or glutathione,



There are exceptions to the above, such as for mixed and multi-ligand groups. For example, histidine, with carboxylic, amino, and imidazole groups, has an intermediate $pK_{a_2} = 6.8$ for the imidazole ring. In general, however, for pH in the range 6–8.5 (approximate pH of a hard water), carboxyl and sulfhydryl groups should be unprotonated, whereas amino, phenolics and alcohols will be protonated. If this is interpreted in terms of charge carboxyl groups and sulfhydryl groups should be negative, phenols and alcohols will be of neutral charge and amino groups should be positively charged.

3. Metal classification

Metals and ligands bind together with bonds ranging from highly ionic to highly covalent and with geometries dependent upon the metal’s position on the periodic table. These ‘species’ may be classified as neutral molecules, ions, ion pairs, clusters, colloids and particles depending upon their size and charge. In nature, the range of ‘soluble’ species is rarely greater than 10 000 Da molecular mass. Instead they are large macromolecules with multi-ligand and multi-metal associations. But to a first approximation, discrete and relatively simple metal–ligand species can be used within an analytical range to describe a natural system.

Various ‘rules’ have been proposed for the coordination of groups of metals to ligand groups. One classification refers to hard metals (Group A) and soft metals (Group B) and their preferred ligands. Hard and soft come from the number of electrons in the outer shell of the metal cation and the polarizability. Hard metals have an inert gas

type (d^{10}) configuration and may be imagined as having spherical symmetry. Thus, adjacent counterions do not deform the electronic field. Soft metals can be deformed and this makes them highly polarizable. In general, without symmetry factors, group B (soft) metals will form stronger bonds with soft ligands under normal environmental conditions. Pearson (1973) has collated and edited a series of benchmark papers on the hard metal and soft metal concept. Table 1 compiles hard, intermediate and soft metals of environmental significance and their preferred ligand associations.

Group B and some intermediate metals may also be ranked on the basis of their electronegativities which are (Stumm and Morgan, 1996): Au(I) > Ag(I) > Hg(II) \approx Cu(I) > Pb(IV) > Pb(II) > Cu(II) > Cd(II) > Co(II) \approx Ni(II) > Fe(II) > Mn(II).

4. Naturally occurring ligands

Both inorganic and organic ligands may be important in metal speciation, depending on the environment and the specific metal. Common inorganic ligands, such as chloride, carbonate, phosphate, hydroxyl and sulfato, associate with Group A metals and have a significant electrostatic bond component. They have been well described, and speciation data for most metals and these ligands can be found in speciation compilations (i.e. Martell and Smith, 2001).

Metal speciation associated with ligands in NOM is poorly known. Here metal speciation with ligands in NOM is developed in context to the

Table 2
Elemental composition ranges for NOM

	% C	% O	% H	% N	% S
HA and HN	50–60	30–35	4–6	2–4	0–2
FA	40–50	55–50	4+	1–3	0–2
Average	52	\approx 40	5.9	2.5	0.7

hard–soft metal classification and ligands that exist in NOM. Emphasis is placed on Group B ligands, specifically metal sulfide clusters (–SM) because silver is expected to associate most strongly with soft ligands.

NOM consists of heterogeneous polydisperse substances of molecular weights ranging from several hundreds to tens of thousands of Daltons. NOM is often divided operationally into humin (HN), humic (HA) and fulvic (FA) acids. In addition, there may be associated inorganic mineral particles and smaller amounts of discrete organic compounds. Bulk NOM is often simply analyzed as soluble organic carbon and particulate organic carbon. The soluble/particulate separation is usually defined by 0.45- μ m size filtration.

The major elemental composition range and average of NOM for HA and FA in freshwaters Thurman (1985) is given in Table 2. NOM concentrations are specified often by weight or by organic carbon. A simple 2:1 ratio of NOM:C can be used to relate the two measurements due to the approximate 50% C content of NOM as shown in Table 2.

Thurman (1985) estimated that oxygen-based functional groups in NOM are approximately carboxyls (2–9%), phenolic (2–6%) and alcoholic

Table 1
Classification of metals of environmental interest and their preferred ligand atoms

Group A metals	Intermediate metals	Group B metals
H ⁺ , Na ⁺ , K ⁺ , Mg ²⁺ , Ca ²⁺ , Sr ²⁺ , Al ³⁺ , Fe ³⁺	Mn ²⁺ , Fe ²⁺ , Zn ²⁺ , Co ²⁺ , Cd ²⁺ , Cu ²⁺ , Pb ²⁺ , Ni ²⁺ , Cr ³⁺ , Fe ³⁺ , Mn ³⁺	Au ⁺ , Cu ⁺ , Ag ⁺ , Cd ²⁺ , Hg ²⁺ , Pb ²⁺ , Sn ²⁺ , Au ³⁺
F > O > N \approx Cl > Br > I > S	<i>with preference for ligand atom</i>	S > I > Br > Cl \approx N > O > F
–OH > –OAr > –OOCR CO ₃ ²⁻ \gg NO ₃ ⁻ PO ₄ ³⁻ \gg SO ₄ ²⁻ \gg ClO ₄ ⁻ particulate oxides	<i>and more specifically</i>	–S, –SR, –SM particulate sulfides
Mainly electrostatic	<i>and nature of bonds</i>	Mainly covalent

(2–4%) hydroxyls, carbonyls (1–5%) and methoxyls (<2%).

Amino acids contribute 15–20% of the nitrogen in aquatic humic substances. These amino acids are structurally part of the humic material bound through amide linkages Thurman (1985). The major amino acids in humic materials are alanine, glycine, aspartic and glutamic acids. In addition to the structural amino acids (amides) there can be additional amino acids and polypeptides associated with the humic substances.

Comparatively, little is known about the forms of sulfur in NOM. Sulfur is usually found in NOM, often at trace (0.1%) levels, but sometimes in the few percent levels. Recently, the occurrence of metastable sulfide clusters has been measured in both oxic and anoxic environments (Bowles et al., submitted for publication, Rozan and Luther, 2002). These clusters may occur discretely, perhaps occupying voids or holes in the NOM. Sulfide groups may also be associated with a metal (e.g. Zn(II)) bound to the S(II⁻) and co-bonded to another ligand in NOM. Xia et al. (1998) used XANES to characterize the oxidation state of sulfur in HAs. They estimated that approximately 50% of the sulfur occurred in reduced oxidation states that would include sulfhydryl, sulfidic and disulfides. They also showed that this component varied markedly for different environments.

Recently, Smith et al. (submitted for publication) demonstrated that Cr(II) reducible sulfide (CRS) correlates closely to total ligands associated with strong binding constants ($\log K' > 11$ for Ag(I)). The hypothesis to be tested further is that CRS is a valid surrogate for –SM ligands, although the CRS method does not determine organic thiols but does include metal disulfides, polysulfides (S_n²⁻) some sulfur (S₈), and partially oxidized S in thiosulfate (S₂O₃²⁻) and sulfites (SO₃²⁻). Bowles et al. (2002a) recently showed that the more commonly measured acid-volatile-sulfide estimates less than 25% of the metal sulfide clusters CuS, Ag₂S, HgS, PbS, CoS and MnS, all of which are potential ligands for Group B metals. CRS, however, quantitatively recovers all of the metal sulfide clusters.

Many intermediate and Group B metals form metal sulfide clusters and nano-crystals. The metal sulfide clusters, by themselves generally net negatively charged (i.e. isoelectric point pH 0.6–3.3, Bebie et al., 1998) seem to bind with other metals and ligands as predicted from the hard–soft metal

associations in Table 1 and chemical coordination principles. Thus, an intermediate metal, Cu(II), e.g., may form a Cu–S structure and at the same time coordinate with a –NH₂ or a –OOC in NOM. In context to possible metal sulfide association with NOM, Dhas et al. (1999) synthesized ZnS nano-particles on SiO₂ microspheres, Brühwiler et al. (1999) produced Ag₂S clusters in zeolite, Trave et al. (1998) made CdS in sodalite, and Gurin (1996) embedded CdS within thiolate cores.

Information on metal sulfide clusters relevant to environmental settings is presently only poorly known for FeS, CuS and ZnS. FeS clusters are complicated and unstable in an oxic environment. They have been described well by Beinert et al. (1998) for cells. CuS, a potentially important and very stable cluster in oxic waters, has been described in part by molecular modeling techniques by Dehnen et al. (1996). ZnS clusters have been synthesized at low micro-molar concentrations and characterized in the laboratory (Bowles et al., 2002a) and shown to stoichiometrically suppress the acute toxicity of Ag(I) to *Daphnia magna* (Bianchini et al., 2002).

Coordination chemistry is important to description of the clusters. For example, an ab initio molecular modeling study of (ZnS)_i, Muilu and Pakkanen (1998) showed no energy discontinuities over the range of $i=3-240$, whereas Matxain et al. (2000) showed a similar continuous energy function for $i=1-9$, based upon ring-like structures.

There are other aspects to be considered for metal speciation in NOM. NOM also associates with ‘insoluble’ inorganic alumino-silicates such as clay minerals. This association can occur in many ways, by anion exchange, chelate formation with Al(III) and Fe(II, III), by chelate formation with transition metal groups, such as copper, and by a ‘water bridge’ involving the water molecule and the COOH. The ‘porous’ nature of NOM allows fixing of other organic molecules such as alkanes, fatty acids, dialkyl phthalates, carbohydrates and peptides as well as metal ions and oxides.

NOM or humic substances contain carboxyl (–OOC), phenolic (–OH), amino (–NHR, –NH₂), sulfhydryl (–SH) and sulfidic (–SM) groups for binding of metal and protons. Acid–base titrations have been used to estimate carboxyl and phenolic functional groups. The pH range used for phenolic estimation, however, may also includes amino

Table 3
Estimates of organic carbon functional group concentrations in natural waters

Environment	DOC mgC/l	Carboxyl ($\mu\text{mol/l}$)	Amino ($\mu\text{mol/l}$)	CRS ($\mu\text{mol/l}$)
Seawater	<0.5	2	0.04	0.0001
Groundwater	0.7	4	0.08	0.0005
River (average)	5	25	0.5	0.015
<i>Lakes</i>				
Oligotrophic	2	1	0.01	0.006
Eutrophic	10	5	1	0.01
<i>Dystrophic</i>				
Lakes	30	15?	1	0.1
Marshes–bog	15–30	<15?	1	0.1

Compiled from Thurman (1985) for carboxyl and amino groups and from Bowles et al. (submitted for publication) for CRS. CRS, reflecting metal sulfide clusters mostly, is used as an estimate of –SM ligands in NOM.

groups (Cox et al., 1999). Better resolution of acid–base titration curves are available to differentiate these species (Brassard et al., 1990), but has not been routinely applied to determine these possible groups.

Table 3 summarizes the range and average DOC for different environments. Total ligand group concentrations are estimated using 5.5 $\mu\text{mol/mgC}$ for carboxyl (–OOC) groups, 0.070–0.2 $\mu\text{mol/mgC}$ for amino (–NH) groups, and 0.003–0.04 $\mu\text{mol/mgC}$ for thiolate (–SR) and metal sulfide (–SM) groups. These ratios can vary a great deal, especially for amino and sulfide ratios. For example, the amino group value can increase 100-fold in an anthropogenically impacted water (DeHaan and DeBoer, 1979), and the thiolate–sulfide ratio will increase in WWTP effluent and anoxic waters. Variations up or down in the functional group concentrations in Table 3 are about a factor of 2 for river water, a factor of 10 for amino groups and a factor of 10 for CRS. Effluent from WWTPs would often be elevated in amino and CRS by a factor of 10 or more from those values given in Table 3.

Metal–ligand associations in NOM are complex. Apparent measured formation constants would be expected to vary with both ligand (functional group) and metal concentrations. Thus, a distribution of binding constants ($\log K'$) with ligand concentrations (L_T), reflecting specific ligand site occupancy, would be anticipated for a specific metal binding in NOM. We can obtain an approximate range of binding constants as a function of

ligand concentration, however, by statistically summing metal formation constants for each functional group in NOM along with the estimated concentrations given in Table 3.

Table 4 summarizes $\log K'$ data for carboxyl, amino and sulfidic–thiolate groups for the major components expected in natural environments and bio-organic matter. This compilation considered 13 carboxylic, 4 amino, 19 amino-carboxylic and 5 sulfidic–thiolate ligand compounds. The ligands tabulated include selections from Hallman et al. (1971) as possible amino acids representative of functional groups in natural biomedica as well as specific groups in NOM as cited by Thurman (1985). These groups in decreasing order of abundance are:

- carboxylic: acetic, formic, butyric, propionic, isovaleric
- amino-carboxylic: glycine, aspartic, glutamic, alanine

Table 4 includes tabulation of the means and standard deviations for $\log K'$ values in model compounds representative of NOM. Formation constants for Table 4 were taken from Martell and

Table 4
Summary of speciation data for carboxyl, amino and sulfide components of NOM

	–OOC, –O	–NH, –OOC	–SR
Na(I)	0.78±0.04 (8)	–	–
K(I)	0.9±0.2 (8)	–	–
Ca(II)	1.6±1.26 (11)	1.63±0.45 (8)	3.15±0.82 (2)
Mg(II)	1.6±1.31 (11)	1.67±0.51 (8)	2.75 (1)
Sr(II)	1.58±0.891 (10)	1.13±0.36 (4)	–
Al(III)	3.74±2.56 (8)	5.56±2.82 (6)	–
Fe(III)	5.43±4.10 (8)	8.45±2.61 (10)	–
Cr(III)	2.13±0.71 (5)	4.47±0.24 (3)	–
Mn(II)	2.27±1.17 (7)	2.80±0.47 (12)	5.6±1.6 (2)
Fe(II)	2.16±1.96 (6)	3.42±0.72 (14)	6.3±0.5 (4)
Co(II)	2.23±1.90 (9)	4.49±0.97 (14)	6.3±2.6 (6)
Ni(II)	5.85±2.38 (8)	5.05±1.56 (22)	6.6±2.6 (6)
Cu(II)	2.01±1.76 (11)	7.34±1.90 (21)	7.6±0.8 (3)
Ag(I)	2.07±1.89 (2)	3.4±0.8 (11)	12.1±1.3 (4)
Hg(II)	5.87±2.61 (7)	7.33±2.35 (8)	16.1±9.2 (3)
Cu(I)	–	4.59±0.97 (4)	14.6±0.8 (3)
Pb(II)	2.55±1.12 (8)	4.53±1.12 (10)	10.2±2.2 (3)
Sn(II)	3.47 (1)	–	–
Cd(II)	2.24±1.39 (8)	3.84±0.75 (13)	8.5±2.0 (4)
Zn(II)	2.19±1.65 (11)	1.40±1.20 (12)	6.8±1.8 (8)

Metal groupings reflect hard, intermediate and soft metals (Table 1). $\log K'$ data shown are mean and standard deviation with sample size for each metal indicated in brackets. Source data for this table is indicated in the text.

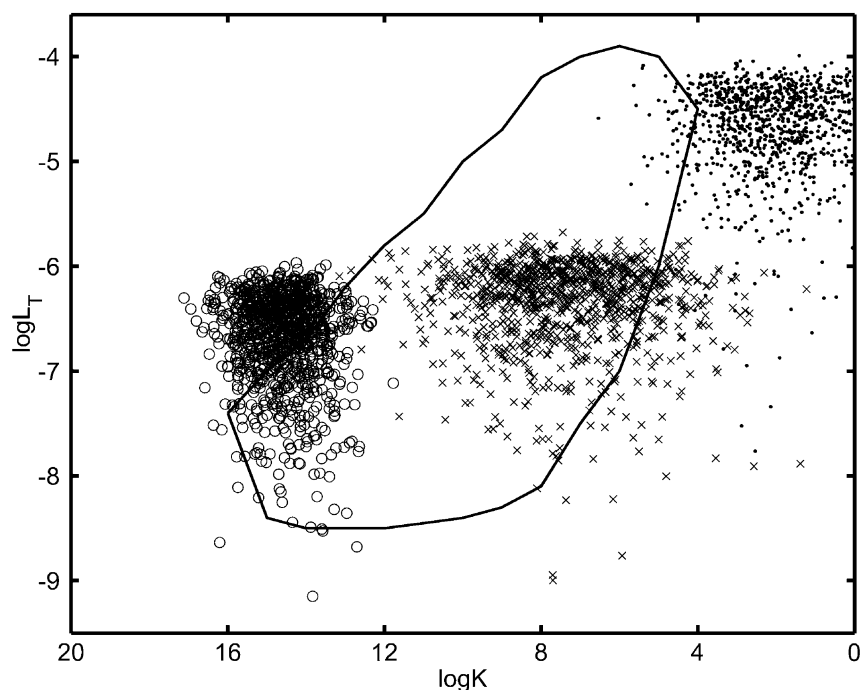


Fig. 1. L_T - $\log K'$ plot for copper. Including carboxylic ligands (\cdot), amino (\times) and sulfide-thiolate (\circ) groups.

Smith (2001) and for sulfides from Al-Farawati and van den Berg (1999), Luther et al. (1996) and for Ag-sulfides from Adams and Kramer (1999a,b). The reduced sulfur compounds selected from Martell and Smith (2001) include: penicillamine disulfide, L-cysteineglycine disulfide, glutathione disulfide, dimethyl sulfide, and the thiols cysteine and glutathione. The lowest ionic strength value was used, and when pH was a variable, the conditional constant was adjusted for a pH of 7. There are no specific data for $-SM'$ or $-SR$ ligands in NOM although indirect evidence suggests that $-SCu$ may be an important ligand near WWTP effluents.

The ranges of concentration, Table 3 and the binding constant data from Table 4 can be combined in a Monte Carlo estimate of the trends in ligand concentration (L_T) and $\log K'$ relationship. Such a plot is shown for copper in Fig. 1 and for silver in Fig. 2.

For both Figs. 1 and 2 the formation constants are generated using the mean and standard deviation from Table 4 for each class of binding site. For Cu binding to reduced sulfur it is assumed that the form of the copper is Cu(I). The concentration ranges selected for each class of ligand correspond to 1–25 μM $-OOC$, 0.01–1 μM

$-NH_2$ and 0.006–0.1 μM $-SR$ and $-SM$ (CRS). The selected concentration range corresponds approximately to range of concentrations observed in natural waters (Table 3). Each range is assumed to represent one standard deviation. Thus, using the means and standard deviations for $\log K'$ and L_T it is possible to generate random simulated data. For both Figs. 1 and 2, one thousand simulations were used. The simulations and plotting were performed using Matlab (The MathWorks Inc., Natick, MA).

As a comparison, Town and Filella (2000) recently compiled a L_T - $\log K'$ plot for various experimental copper speciation studies. The results of the compilation of Town and Filella (2000) are shown as a solid outline on Fig. 1. It can be seen that the summary of model ligands presented here and the summary of Town and Filella (2000) are very similar. In particular, more than half of the simulated amino and sulfide-thiol ligands fall within the range of experimental values summarized by Town and Filella (2000). There are fewer carboxyl ligand represented within the range of experimental values though. This is likely because experiments would have to be performed at higher total copper than is generally used in order to study these relatively weak binding sites. In addi-

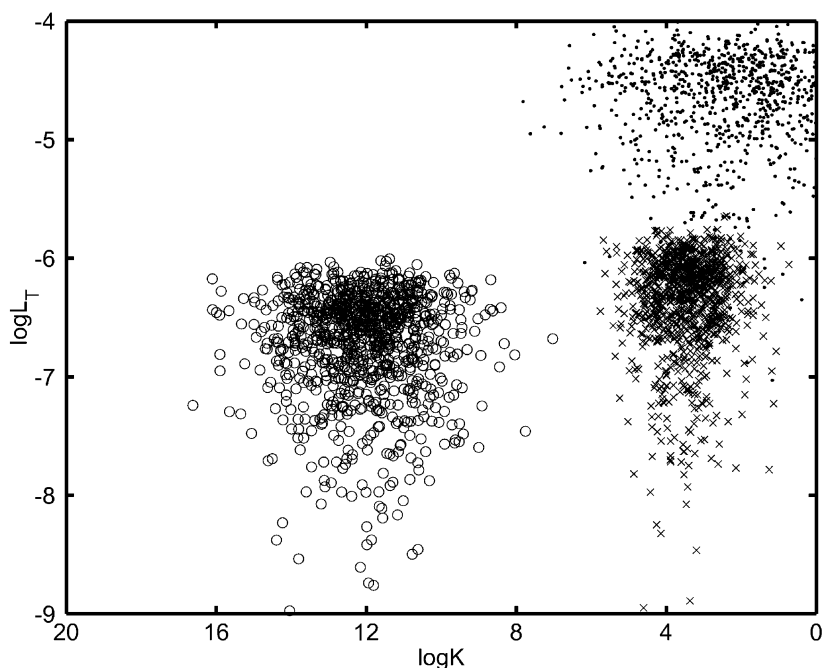


Fig. 2. L_T - $\log K'$ plot for silver. Including carboxylic ligands (\bullet), amino (\times) and sulfide-thiolate (\circ) groups.

tion, the simulated carboxylic sites are for monodentate complex formation and often carboxylic sites within NOM are thought to be bidentate (Tipping, 1998). Overall, it seems reasonable that one may use the approach of summarizing $\log K'$ values for model compounds to represent a first approximation of NOM. As more information becomes available then data in Tables 3 and 4 may be updated.

As a comparison to Cu(II) a L_T - $\log K'$ plot is shown for Ag(I) in Fig. 2. Comparison of Figs. 1 and 2 shows that the distribution of binding strengths is much more discrete for ligands binding silver compared to copper. The copper binding constants show a pseudo-linear trend where the values for the different types of ligands show some overlap. The binding constants for silver to the same ligands show essentially no overlap between the different classes of ligands. The differences result from the fact that silver has such a strong affinity for reduced sulfur and a relatively weak affinity for nitrogen and particularly oxygen.

5. Discussion

NOM is an extremely important and complicated substrate for metal binding. As a first approximation, discrete functional group concentrations

and formation constants for model ligands may be used statistically to obtain an L_T - $\log K'$ plot. Such a plot can be used to simulate metal speciation over a range of concentrations for initial predictions of speciation and as a tool to design speciation experiments.

This study emphasizes the importance of thiolate-metal sulfide clusters as key ligands to the more toxic Group B metals and some intermediates. We also indicate that CRS measurement agrees well with the few speciation studies at these low concentrations. This is further confirmed when the L_T - $\log K'$ plot generated for copper, is compared to a literature compilation. The approach proposed here may provide the best estimate for L_T - $\log K'$ plots for other metals as experimental data is often lacking.

Many studies on toxicity consider the effect of other parameters, often hardness, on the suppression of toxicity. One may use the metal-ligand classification proposed here to examine these and other relationships further. For example, if one considers the suppression (or enhancement) of toxic effect of a Group A metal due to hardness (Group A metals), then one may postulate a metal competition effect for the same ligand site. But if one considers suppression of a Group B metal by

hardness (Group A metals), then separate ligands or different ligand sites on a complex molecule might be hypothesized.

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