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## Laboratory measurements and modeling of metal–humic interactions under estuarine conditions

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**Abstract**—Equilibrium dialysis was used to measure Co- and Cu-binding by an isolated peat humic acid (PHA) in controlled laboratory experiments under simulated estuarine conditions: ionic strengths of 0.005 to 0.7 M in NaCl and mixed Na-Mg-Ca chloride solutions, with trace metal concentrations of  $\sim 5 \times 10^{-7}$  M, a PHA concentration of 10 mg/L, and at constant pH values of  $\sim 7.8$  (Co and Cu) and  $\sim 4.6$  (Cu only). Generally, Co- and Cu-humic binding decreased substantially with increasing ionic strength and, in the case of Cu, with decreasing pH. The presence of seawater concentrations of Ca and Mg had a relatively small effect on Co-humic binding and no measurable effect on that of Cu under the experimental conditions. The binding data were well-described by an equilibrium speciation code (the Windermere Humic Aqueous Model, WHAM) after optimising the fits by varying the metal–proton exchange constants for humic acid within justifiable limits (i.e., within 1 standard deviation of the mean exchange constants used in the WHAM database). The main factor producing the observed variations in metal–humic binding at constant pH was the electrostatic effect on the humic molecule. WHAM was used to predict Co- and Cu-humic binding in simulations of real estuaries. Co-humic binding is predicted to be relatively unimportant (generally  $< 5\%$  of total Co), whereas the Cu-humic complex is likely to be the dominant species throughout an estuary. The main factors producing changes in Co- and Cu-humic binding in the real-estuary simulations are the electrostatic effect on the humic molecule, ligand competition (mainly from carbonate species) for metals, and to a lesser extent Ca and Mg competition for humic binding sites. Variations in pH are significant only at the freshwater end of an estuary. WHAM simulations also indicated that competition effects between metals are more likely to occur in freshwaters than in seawater, due to enhanced electrostatic binding at low ionic strength. Copyright © 2002 Elsevier Science Ltd

### 1. INTRODUCTION

Humic substances are present in most surface and ground waters and are important with respect to the chemical speciation, mobility, and bioavailability of trace metals. Substantial advances have been made in recent years in modeling metal–humic interactions so that a number of models now exist that are capable of predicting metal–humic binding under a range of solution conditions, e.g., Humic Ion-Binding Models V (Tipping and Hurley, 1992) and VI (Tipping, 1998), and the non-ideal competitive adsorption (NICA)–Donnan Model (Benedetti et al., 1995). There are considerable published experimental data on proton binding at various ionic strengths with which to calibrate the proton dissociation and electrostatic components of these models (Tipping and Hurley, 1992). There are also sufficient metal–humic binding data with which to undertake an initial calibration of the metal–proton exchange reactions (Tipping and Hurley, 1992). However, there are little or no experimental data on metal–humic binding, directly involving both variations in ionic strength and concentrations of competing major cations, suitable for validating the models under estuarine conditions. Estuaries are ideal for testing speciation schemes, such as Model V, because the factors that most affect metal–humic interactions exhibit a wide range of parameter values therein, with the possible exception of pH.

The most important are likely to be competition effects from Ca and Mg, the effect of ionic strength ( $I$ ) on electrostatic interactions between metals and humics, and the indirect effect of ligand competition from the major inorganic anions.

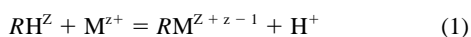
Metal–humic interaction in estuaries is a topic of considerable interest, linked particularly to questions concerning the fate of pollutants and of riverborne weathering products. Current ideas concerning metal–humic interactions in estuaries, described in standard oceanographic texts (Chester, 1990; Libes, 1992), originate mainly from the study of Mantoura et al. (1978). Most importantly, competition from Ca and Mg is thought to be the main controlling effect, resulting in decreased humic-binding of trace metals under more saline conditions. Ca and Mg were predicted to occupy  $> 99\%$  of the humic binding sites under seawater conditions and, as a result, the complexation of Cu, for example, was predicted to fall from  $> 90\%$  to  $\sim 10\%$  going from freshwater to seawater (Mantoura et al., 1978). More recent predictions using Model V (Tipping et al., 1998) have suggested that Ca and Mg competition effects are less important in estuaries than previously thought. Additionally, recent laboratory experiments have demonstrated the potential importance of ionic strength (i.e., the electrostatic effect) on metal binding by humic substances (Brown et al., 1999; Pinheiro et al., 1999). The initial aims of the present work therefore were (i) to determine experimentally the effects of ionic strength and Ca and Mg competition on the humic complexation of strongly (Cu) and weakly (Co) binding metals under simulated estuarine conditions, and (ii) to use the data to

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test Humic Ion-Binding Model V. After successful testing, the model was then used to predict humic binding of Cu and Co in simulations of real estuaries with contrasting freshwater pH values (5.1 and 7.8) and to explore the role of Ca and Mg competition more generally in natural waters.

## 2. HUMIC ION-BINDING MODEL V, WHAM, AND BINDING UNITS

In this work, the speciation of dissolved metals is computed with the Windermere Humic Aqueous Model, WHAM (Tipping, 1994). This model takes into account the interactions of metals with (i) inorganic ligands ( $\text{OH}^-$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ), using conventional equilibrium formulations and equilibrium constants from the literature, and (ii) humic substances using Humic Ion-Binding Model V (Tipping and Hurley, 1992). The essential features of Model V are as follows. Humic compounds are represented by hypothetical size homogeneous molecules, which carry proton-dissociating groups that can bind metal ions. The interactions are described in terms of intrinsic equilibrium constants, which refer to the (usually hypothetical) situation where the humic substances have zero electrical charge, and an electrostatic term, which takes into account the influence on binding of the variable humic charge. Two types of proton-binding groups are recognised, one (type A) being relatively acid (mainly carboxyl groups) and the other (type B) less acid (e.g., phenolic groups). Each type is heterogeneous, comprising four sites with equally spaced  $\text{pK}$  ( $-\log_{10} K$ ) values that are defined by two adjustable parameters (median and spread values obtained by fitting). Metal binding takes place at single proton-binding sites (monodentate) and at bidentate sites formed by pairs of proton-dissociating sites. The model permits the binding of the first hydrolysis product (e.g.,  $\text{CuOH}^+$  in the case of  $\text{Cu}^{2+}$ ) as well as the parent species. The binding is characterised by intrinsic equilibrium constants ( $K_{MHA}$  and  $K_{MHB}$ ) for metal-proton exchange reactions of the type:



where  $R$  is the humic molecule,  $Z$  is the charge on the humic molecule ( $\text{eq g}^{-1}$ ), and  $z$  is the charge on the metal ion ( $M$ ). A representative equilibrium constant expression, together with its appropriate electrostatic correction, is given by:

$$\frac{[RM^{Z+z-1}] a_{H^+}}{[RH^Z] a_{M^{z+}}} = K_{MHA} e^{-2wZ(z-1)} \quad (2)$$

where  $[ ]$  = concentrations and  $a$  = activities (both in  $\text{mol L}^{-1}$ ) in the bulk solution, and  $w$  is the electrostatic interaction factor, given by:

$$w = P \log_{10} I \quad (3)$$

where  $P$  is an empirical proportionality constant, included in the WHAM database, and  $I$  is the ionic strength. The default value of  $P$  was estimated by fitting literature data of proton binding undertaken at variable  $I$  (Tipping, 1994). The exponential (electrostatic) term in Eqn. (2) arises from the theoretical treatment of electrostatic effects given by Tanford (1961), derived by applying the Debye-Hückel model to macromolecules.

During the early stages of calibrating Model V it was found that values of  $\text{pK}_{MHB}$  vary linearly with  $\text{pK}_{MHA}$ , so that it is possible to characterise metal binding with a single parameter,  $-\log_{10} K_{MHA}$  or  $\text{pK}_{MHA}$  (Tipping, 1994). The strength of metal-humic binding therefore increases with decreasing values of  $\text{pK}_{MHA}$ . The linear relationship is consistent with observations for the metal complexes of simple carboxylic and phenolic ligands, as shown by Martell and Hancock (1996). The WHAM database contains default values of  $\text{pK}_{MHA}$  for 31 metals, estimated from literature data or by linear free-energy correlations. The (nonspecific) accumulation of an excess of counterions in the diffuse layer adjacent to the molecular surface also contributes to the total binding.

WHAM was originally designed for freshwaters and soils, and calculates activity coefficients for ions with the extended Debye-Hückel equation, which is applicable only up to an ionic strength of  $\sim 0.1$  mol/L. For this study, WHAM was modified to allow the use of the Davies equation, which is valid up to an ionic strength of around 0.5 mol/L (Stumm and Morgan, 1996). The use of the Davies equation will therefore result in some errors in the prediction of metal speciation in full-strength seawater, although the significance of these errors will be minor in the context of the observed and predicted ionic strength trends. Within WHAM, Model V was used as the humic submodel in preference to Model VI (Tipping, 1998) because of current uncertainties of how best to deal with the competitive effects of Fe(III) and other strong binding metals, present at unknown concentrations (Bryan, 2001; Peters et al., 2001). Model VI (Tipping, 1998) was developed from Model V to provide a better description of published binding data obtained at low [metal]:[humic] ratios. Model VI differs from Model V in having an extended range of binding site strengths at low  $\text{pK}_{MHA}$  values, resulting in a relatively small number of particularly strong binding sites. The significance of this choice of model to the interpretation of our results is considered in Section 5.4.

Metal-humic binding is expressed as % metal bound and as  $\nu_M$ , the total amount of metal specifically bound to the humic molecule ( $\text{mol g}^{-1}$ ). The term  $\nu_M$  is comparable to  $[RM^{Z+z-1}]$  in Eqn. (2) divided by the concentration of humic substances ( $\text{g L}^{-1}$ ), but it relates to the total amount bound by all the heterogeneous monodentate and bidentate sites. Methods used for characterising metal binding commonly have a limited analytical window (Town and Filella, 2000). The dialysis method employed in the present study cannot generally be used much outside the range 10 to 90% humic-bound because of problems associated with quantifying whichever is the smaller, the humic-bound fraction or the nonbound fraction (Lead et al., 1998). Consequently, it was necessary to obtain a spread of binding values within this range to provide a reliable test of WHAM. In the case of Cu, this required a series of binding experiments at a relatively low pH ( $\sim 4.5$ ) (see Section 3). It follows from the law of mass action (see Eqn. 2) that the percentage bound between 10 and 90% will be highly sensitive to pH and the  $\text{pK}_{MHA}$ , so that humic binding within this range also provides a rigorous test of the model. Illustrative WHAM simulations, at total metal concentrations of  $10^{-6}$  M and a humic acid concentration of 10 mg/L in 0.01 mol/L NaCl, indicate that the range 10 to 90% humic-bound can be brought about by changes in pH of 1.0 (pH 3.6 to 4.6) for Cu and 1.4 (pH 5.2 to 6.6) for Co, or changes in  $\text{pK}_{MHA}$  of 0.6 ( $\text{pK}_{MHA}$  1.25 to 1.85) for Cu and 1.1

Table 1. The measured pH (mean and standard deviation) in the binding experiments, with the numbers of experimental replicates shown in parentheses.

Experimental series <sup>a</sup>						
<i>I</i> (/M)	Cu/Na low pH	Cu/Na high pH	Cu/NaMgCa low pH	Cu/NaMgCa high pH	Co/Na	Co/NaMgCa
0.005	4.57 ± 0.04 (3)	7.79 ± 0.12 (3)	4.58 ± 0.01 (5)	8.07 ± 0.02 (6)	7.92 ± 0.03 (6)	8.03 ± 0.03 (6)
0.05	4.38 ± 0.03 (3)	— (0)	4.62 ± 0.01 (4)	— (0)	7.77 ± 0.02 (6)	7.87 ± 0.05 (6)
0.15	4.69 ± 0.14 (9)	7.84 ± 0.01 (2)	4.51 ± 0.02 (4)	7.82 ± 0.00 (3)	7.82 ± 0.02 (5)	7.76 ± 0.06 (5)
0.35	4.56 ± 0.03 (3)	7.79 ± 0.06 (5)	4.44 ± 0.05 (9)	7.93 ± 0.15 (3)	7.70 ± 0.02 (4)	7.79 ± 0.01 (6)
0.7	4.46 ± 0.09 (7)	7.73 ± 0.03 (3)	4.51 ± 0.01 (3)	7.71 ± 0.02 (4)	7.68 (1)	7.68 ± 0.07 (6)

<sup>a</sup>Experiments in NaCl solutions indicated by /Na, those in mixed NaCl, MgCl<sub>2</sub>, CaCl<sub>2</sub> solutions by /NaMgCa.

( $pK_{MHA}$  2.2 to 3.3) for Co. The variable pH simulations were undertaken using the default  $pK_{MHA}$  values for humic acid (1.5 for Cu and 2.7 for Co). The variable  $pK_{MHA}$  simulations used the pH values (4.1 for Cu and 6.0 for Co) at which 50% of each metal is humic bound with the default  $pK_{MHA}$  values.

### 3. METHODS

#### 3.1. Collection and Isolation of Humic Sample

Approximately 1 kg of moorland peat was collected from Whitray Fell, North Yorkshire (Lat. 54°5' N, Long. 2°30' W), at the same location as used in a previous study (Lead et al., 1994). A peat humic acid (PHA) fraction was extracted as described by Reid et al. (1990). Briefly, the peat was wet sieved (4-mm mesh) and then treated with nitrogen-saturated NaOH (0.1 mol/L Na in final solution). After centrifuging and discarding the residue, the supernatant was acidified to pH 2 using concentrated HCl. The resulting suspension was left for 2 d in the dark to allow complete precipitation of humic acid and then the suspension was re-centrifuged and the supernatant discarded. The resulting PHA sample was subjected to successive treatments in nitrogen-saturated 1 mol/L KOH, HCl (pH 2), and a 0.3 mol/L HF–0.1 mol/L HCl mixture to remove bound metals and dissolve, as far as possible, any residual mineral content. Finally the humic acid residue was dialysed (Visking tubing) against Milli-Q water until chloride was undetectable, and then freeze-dried. Before the experiments, 1 g/L stock solutions of PHA were prepared in Milli-Q water and stored in the dark at 4°C.

#### 3.2. Experimental Protocol

Metal–humic binding was determined by equilibrium dialysis, following a similar approach to that described by Lead et al. (1998). The experiments were carried out in 1 L high-density polyethylene (HDPE) screw top bottles, using Spectra/Por cellulose ester dialysis tubing (2000 molecular weight cut-off) that had been thoroughly prewashed in Milli-Q water. Preliminary experiments were carried out to determine equilibration times (~24 h) for Cu and Co through the membranes, under the solution conditions used in the main experiments.

Two simple analog solutions of estuarine systems were prepared using AnalaR reagents, one containing NaCl only and the other containing NaCl, MgCl<sub>2</sub>, and CaCl<sub>2</sub> in their sea water ratios (i.e., Na: Mg:Ca molar ratios of 45.6:5.4:1). Cu and Co binding were studied independently in each medium at five different ionic strengths: 5 mM, 50 mM, 0.15 mol/L, 0.35 mol/L, and 0.7 mol/L. The solutions were spiked with Perkin Elmer acidic metal standards to give final concentrations of  $4.7 \times 10^{-7}$  M Cu and  $5.1 \times 10^{-7}$  M Co. AnalaR NaOH (0.1 mol/L) and HNO<sub>3</sub> (10% by vol.) were added as required to adjust the pH of the solutions. Experiments were undertaken at a single narrow pH range (~7.7 to 8.0) in the case of Co and at two narrow pH ranges (~4.4 to 4.7, 7.7 to 8.1) in the case of Cu. The high pH solutions were equilibrated with atmospheric CO<sub>2</sub> by bubbling with water-saturated air for 24 h. PHA was added from the 1 g/L stocks to give a final humic concentration of 10 mg/L. A length (~20 cm) of dialysis tubing was filled with the relevant trace-metal free, ionic medium, then sealed and

inserted into each experimental solution. Experiments were allowed to reach dialysis equilibrium over 4 d with continuous stirring. Each batch of experiments was run with a control, containing no PHA, to confirm that dialysis equilibrium was achieved for both Co and Cu. Typically, three to five replicate binding experiments were run under each set of conditions (Table 1).

After 4 d, the experiments were terminated and samples taken from the bulk solution and from inside the dialysis tubing. Metal complexation reactions with humic substances are generally regarded as being fast (minutes) (Lin et al., 1994), although complete equilibration may take in the order of 24 h (Ma et al., 1999). Therefore chemical equilibrium, as well as dialysis equilibrium, would be expected. The samples for trace metal analysis were then acidified to pH 2 with 10% HNO<sub>3</sub>. A separate sample from inside the tubing was checked for leakage of PHA in every experiment by measuring the absorbance at 340 nm after mixing with an equal volume of pH 7 buffer. Subsequent calculations of metal binding were corrected for any leakage, assuming that the leaked PHA had the same binding affinity as the bulk PHA. The estimated leakage effects, equivalent to mean and median absolute errors of 1.8% and 0.5% in the calculated percentage bound, were generally less than the experimental uncertainties (see error bars in Fig. 1). The final pH of the bulk solution was also measured. Trace metal analysis was carried out by graphite furnace atomic absorption spectrometry (GFAAS) using a matrix modifier in the more saline solutions and matrix matched standards. The modifier consisted of a 550 ppm solution of palladium nitrate in 1% hydroxyammonium chloride and 1% nitric acid (A. Tappin, personal communication).

### 4. RESULTS AND WHAM PREDICTIONS

In the NaCl medium at a pH of ~7.8, Co–humic binding decreased markedly with increasing ionic strength (Fig. 1a), whereas Cu remained predominantly bound to PHA at all ionic strengths (Fig. 1c). The presence of Ca and Mg decreased Co binding still further (Fig. 1b), whereas no significant change in Cu binding was apparent in the mixed salt medium at high pH (Fig. 1d). At low pH, in contrast, Cu exhibited a marked decrease in binding with increasing ionic strength (Fig. 1e), but the effect of Ca and Mg was again small if not negligible (Fig. 1f).

WHAM simulations were run for each set of experimental conditions, including the mean measured pH values (Table 1), with the Model V parameters initially set to their default values for humic acid. Given that binding is highly sensitive to pH and  $pK_{MHA}$  within the range 10 to 90% bound (see Section 2), these blind predictions were good in the sense that the general scale of binding and the relative effects of variable *I* and variable Ca, Mg concentrations were reproduced in all cases (Fig. 1a–f).

With the assumption of a fixed relationship between  $pK_{MHA}$  and  $pK_{MHB}$ , the former is the only parameter in Model V that directly affects metal binding. The default values of all the

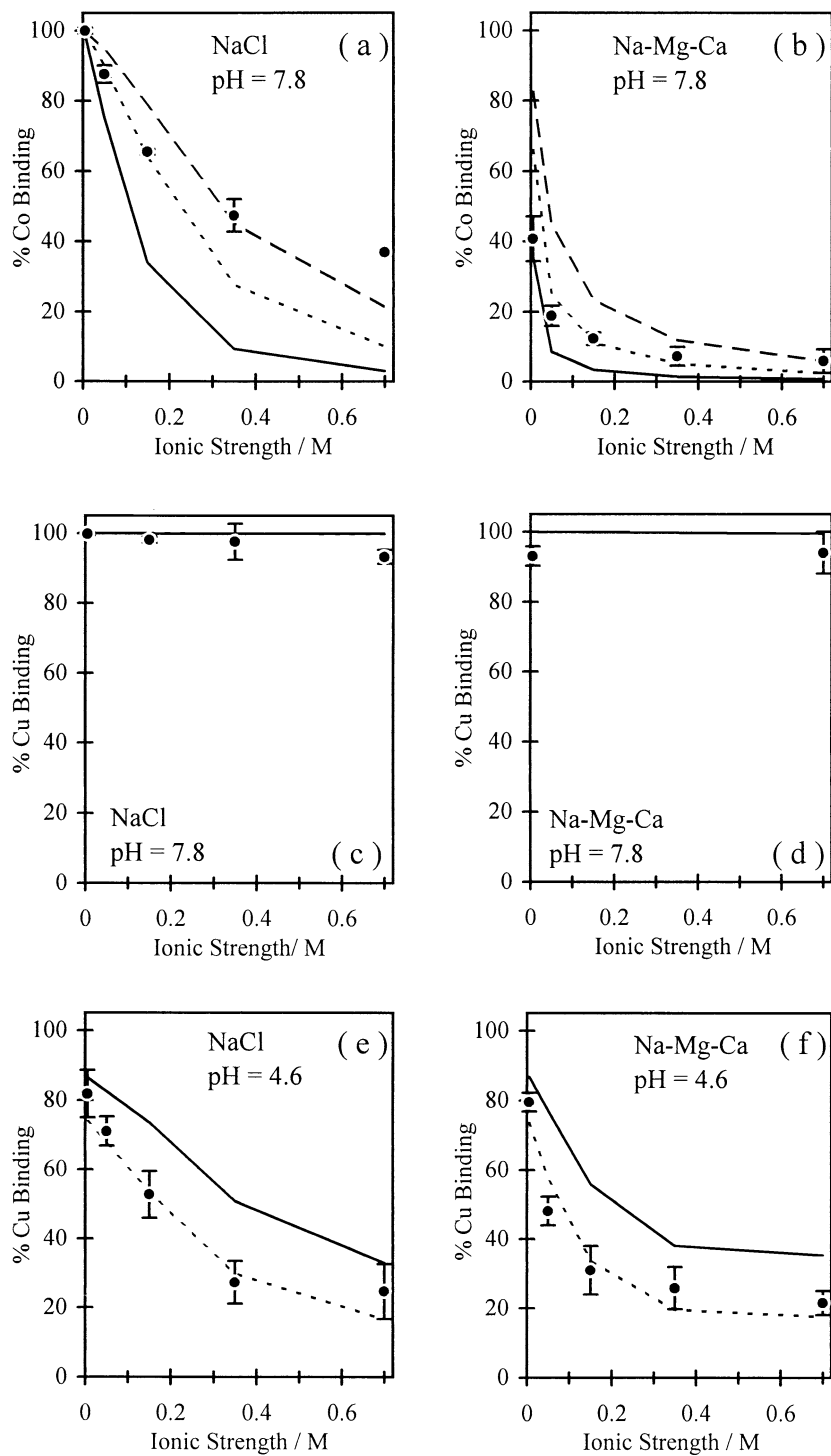


Fig. 1. Metal-humic binding results of experiments, WHAM predictions using default database (—), and WHAM fits following optimization of  $pK_{MHA}$  values (--- fitted to NaCl data only; ..... fitted to data for both ionic media). (a) Co in NaCl solution at pH  $\sim$ 7.8. (b) Co in Na-Mg-Ca chloride solution at pH  $\sim$ 7.8. (c) Cu in NaCl solution at pH  $\sim$ 7.8. (d) Cu in Na-Mg-Ca chloride solution at pH  $\sim$ 7.8. (e) Cu in NaCl solution at pH  $\sim$ 4.6. (f) Cu in Na-Mg-Ca chloride solution at pH  $\sim$ 4.6. Error bars represent the standard deviations of experimental replicates.

Table 2. A comparison between the optimised  $pK_{MHA}$  values in this study with the default values in the WHAM database.

	$pK_{MHA}$	Range	Stand. dev.	No. of datasets
Cu (optimised)	1.61	—	—	1
Cu HA (WHAM)	1.5	1.1–1.8	0.4	4
Cu FA (WHAM)	0.8	0.6–0.9	0.1	6
Co (optimised)	2.45 and 2.55	—	—	1
Co Ha (WHAM)	2.7	1.9–3.8	1.0	3
Co FA (WHAM)	1.7	1.3–1.9	0.3	6

other Model V parameters, including the electrostatic parameter  $P$  in Eqn. (3), were obtained independently by fitting the large number of published data sets on proton binding by humic and fulvic acids (Tipping and Hurley, 1992). Therefore the default predictions shown in Figure 1 can only justifiably be improved on the basis of optimising the  $pK_{MHA}$  values. The strategy adopted was to minimise the sum of the squared errors in percentage metal bound by optimising the  $pK_{MHA}$  values for Cu and Co, leaving the Ca and Mg values at their default settings. In the case of Cu, the minimisation routine was applied simultaneously to the low pH data for both ionic media with excellent results (Fig. 1e,f). Cu-humic binding at high pH (Fig. 1c,d) was predicted using the optimised  $pK_{MHA}$  value but was not used in the fitting because all the data points fell outside the effective analytical window of the dialysis method (10 to 90% binding). In the case of Co, two approaches were adopted. The first involved simultaneously fitting the data for both ionic media, as with Cu, while the second involved fitting the data for the NaCl medium alone (Fig. 1a) followed by the prediction of binding in the mixed salt solution (Fig. 1b). Both approaches gave better fits than that based on the default values, but neither was as good as that for Cu. Table 2 shows that the optimised  $pK_{MHA}$  values of 1.61 for Cu (both media), and 2.55 (both media) and 2.45 (NaCl) for Co are all well within a single standard deviation of the WHAM default values for humic acid, based on the published data used to derive them (Higgo et al., 1993; Tipping, 1993).

## 5. DISCUSSION

### 5.1. Ionic Strength Dependence of Metal–Humic Binding in NaCl solution

Given the success of WHAM in predicting the experimental data, using acceptable  $pK_{MHA}$  values, and given that the model is based on established chemical principles, WHAM can justifiably be used to explore the nature of the observed humic binding–ionic strength relationships. The fact that Cu remained predominantly bound to PHA at all ionic strengths at pH  $\sim 7.8$  in NaCl solution (Fig. 1c) suggests that Cu-humic binding is insensitive to  $I$  under these conditions, compared with the binding observed in the other two series of experiments undertaken in NaCl solutions (Fig. 1a,e). Examination of the WHAM output data, however, shows that this conclusion is potentially misleading. Tables 3–5 show selected output from the various WHAM calculations for the binding experiments in NaCl solutions, including  $\nu_M/a_{M^{2+}}$  values. The WHAM output also indicated that diffuse layer binding of Cu and Co was negli-

ble in all experiments, so that  $\nu_M \approx$  total bound metal. The relative values of  $\nu_{Cu}/a_{Cu^{2+}}$  shown in Tables 3–5 indicate that Cu-humic binding in NaCl at high pH is just as sensitive to  $I$  as humic binding in the other two series of experiments in NaCl solution (Cu at low pH and Co at high pH). In fact, using  $\nu_{Cu}/a_{M^{2+}}$  as the measure of binding indicates that it is Cu-humic binding at low pH that is the least sensitive to  $I$  (Table 5). The fact that such a large change in  $\nu_{Cu}/a_{M^{2+}}$  could not be confirmed by the experimental measurements is attributable to the overall strength of Cu-humic binding at circum-neutral pH, resulting in the percentage binding being  $>90\%$  in all cases and therefore outside the analytical window. It was only at low pH, where Cu-humic binding is much weaker, that a much smaller change in the  $\nu_{Cu}/a_{M^{2+}}$  ratio was reflected in a measurable change within the range 10 to 90% bound.

The factors causing the observed dependence of metal–humic binding on  $I$  in NaCl solution potentially include competition for humic binding sites from  $Na^+$ , changes in free metal ion activities due to chloride and carbonate complexation, an electrostatic effect on the humic molecule (see Eqns. 2 and 3), and a decrease in the activity coefficient of the free metal ions with increasing  $I$ . Monovalent metals like  $Na^+$  have far less affinity for humic functional groups than polyvalent metals (Bonn and Fish, 1993), and it is for this reason that, in Model V, monovalent metals are assumed to bind only non-specifically as counterions in the diffuse layer (Tipping and Hurley, 1992). The success of the model in predicting the observed binding behaviour of Cu and Co is consistent with the lack of any substantial specific  $Na^+$  binding.

Total carbonate and bicarbonate species were predicted to be present at maximal concentrations of 12% and  $<1\%$  for Co and Cu, respectively, and in the case of Co the maximal concentrations of these species occurred at an intermediate ionic strength of  $\sim 0.15$  mol/L. Therefore in terms of both absolute concentrations and their ionic strength trends, carbonate species cannot account for the observed binding trends. Chloride complexation is clearly significant in both the Co and low pH, Cu experiments, varying from  $<1$  to  $\sim 20\%$  of the total metal species (Tables 3 and 5). The activity coefficients of the free ions of both Co and Cu are predicted to decrease by a factor of  $\sim 3$  with increasing  $I$  from 0.005 to 0.7 mol/L. Therefore, at first appearance, chloride complexation and simple activity coefficient effects both appear to be important in explaining the metal–humic binding trends shown in Figures 1a and 1e. However, the predicted free metal ion activities either vary little with  $I$  (Cu at low pH, Table 5) or actually increase over all or part of the range of increasing  $I$  (Cu at high pH, Table 4; Co, Table 3), indicating that chloride complexation and the varying activity coefficients are not the overriding factors controlling the metal–humic binding.

The electrostatic effect on the humic molecule results in decreased attraction, and hence binding, between metals and the negatively charged humic molecule with increasing  $I$ . The effect is ascribed to the increased shielding effect of the solution ions and to the associated decreased diffuse-layer thickness. The scale of the effect within each of the three experimental series can be assessed by running WHAM under the extreme ionic strength conditions with fixed values of  $a_{M^{2+}}$  and pH. It follows from Eqn. (2) that the only factors then affecting the amount of specifically bound metal are the exponential term

Table 3. Selected WHAM output data for Co binding experiments in NaCl solutions at pH ~7.8.

<i>I</i> (M)	$\nu_{\text{Co}}(\text{mol g}^{-1})/10^{-6}$	$\frac{\nu_{\text{Co}}}{a_{\text{Co}^{2+}}}(\text{Lg}^{-1})$	$a_{\text{Co}^{2+}}$ (nM)	$\gamma_{\text{Co}^{2+}}$	[Co <sup>2+</sup> ] (nM)	[CoCl <sup>+</sup> ] (nM)	[CoCl] (as % of total Co)	[Co <sup>2+</sup> ]
0.005	53	25,000	2.1	0.73	3	0.02	0.004	0.6
0.05	39	910	43	0.47	95	4.2	0.82	19
0.15	17	220	79	0.31	250	23	4.6	50
0.35	4.9	56	88	0.25	360	60	12	70
0.7	1.5	19	80	0.23	350	110	22	68

$\nu_{\text{Co}}$  = concentration of Cu specifically bound to humics; [] denotes solution concentrations;  $a_{\text{Co}^{2+}}$  = free ion activity;  $\gamma_{\text{Co}^{2+}}$  = activity coefficient of free ion.

and the degree of protonation of the humic molecule, which together can be considered as constituting the electrostatic effect on metal binding. As ionic strength increases, protonation decreases due to increased shielding and therefore the negative charge on the humic molecule also increases. The increase in negative charge has the effect of counteracting to some degree the effect of increased shielding on metal ion binding. To obtain fixed values of  $a_{\text{M}^{2+}}$  and pH, the total trace metal concentrations at  $I = 0.7$  mol/L were varied until WHAM gave the same  $a_{\text{M}^{2+}}$  values as those predicted at  $I = 0.005$  mol/L with the original total trace metal concentrations ( $4.7 \times 10^{-7}$  M Cu and  $5.1 \times 10^{-7}$  M Co). Table 6 shows the final values of variables used in the calculations. The predicted differences in  $\nu_{\text{M}}/a_{\text{M}^{2+}}$  between the 0.005 and 0.7 mol/L solutions of each series (Table 6), at fixed values of  $a_{\text{M}^{2+}}$  and pH, are similar to those predicted under the experimental conditions (Tables 3 to 5). It follows that the electrostatic (coulombic) effect is the dominant factor underlying the observed variations in metal–humic binding in NaCl solutions.

Although the electrostatic submodel within Model V provides a good description of the dependence of metal–humic binding on ionic strength, observed in this and a limited number of other studies (Higgo et al., 1993; Tipping and Hurley, 1992), alternative electrostatic models have been used for humic substances (Bartschat et al., 1992; Benedetti et al., 1995). Given the apparent importance of the electrostatic term for metal–humic interactions (see also Sections 5.3 and 5.4), additional measurements of ionic strength effects would enable a proper comparison of the performance of these various alternative models.

## 5.2. Metal–Humic Binding in the Presence of Ca and Mg

The results shown in Figure 1 indicate that sea-water concentrations of Ca and Mg have a small competitive effect on

Co-binding by PHA but a negligible effect on that of Cu under the conditions employed in our experiments. The findings for Cu contrast with the combined experimental-modeling study of Mantoura et al. (1978) that predicted a decrease in humic binding from ~100% to ~10% down estuary due principally to competition from Ca and Mg. In their study, Mantoura et al. (1978) isolated humic material from various sources, determined conditional metal–humic association constants ( $K_{\text{c}}$ ) at a single set of conditions (pH 8.0 and  $I = 0.02$  mol/L), and used these constants to make predictions of metal speciation under estuarine conditions using an equilibrium speciation code based on HALTAFALL (Ingri et al., 1967). Thus competition by Ca and Mg was not measured directly.

The contrasting Cu-binding characteristics reported in this and the Mantoura et al. (1978) studies may be related to a number of factors, the most obvious of which are the binding strengths of the humics and the concentrations of humic binding sites and metals. The equilibrium constants cannot be compared directly between the two studies because the metal–humic interactions are expressed as metal association reactions in the Mantoura model and by metal–proton exchange reactions in Model V (Eqn. 1). However, a measure of the relative binding strengths was obtained by comparing the ratios of the equilibrium constants for Ca, Mg, Cu, and Co (Table 7). Trace metal binding occurs principally at bidentate sites in the Model V framework (Tipping et al., 1998) and the bidentate constants are obtained by multiplying the relevant monodentate constants for each metal (i.e. adding the p*K* values) (Tipping, 1994). The comparison therefore used the p*K*<sub>MHA</sub> value for each metal multiplied by 2, corresponding to bidentate binding by two carboxyl groups. This analysis yielded the same order of binding strength in both studies (Cu ≫ Co > Ca ≈ Mg) but in different ratios, i.e., 3200:13:1 with Model V and 250,000:8:1 in the Mantoura study. The latter ratios were obtained using the

Table 4. Selected WHAM output data for Cu binding experiments in NaCl solutions at pH ~7.8.

<i>I</i> (M)	$\nu_{\text{Cu}}(\text{mol g}^{-1})/10^{-6}$	$\frac{\nu_{\text{Cu}}}{a_{\text{Cu}^{2+}}}(\text{Lg}^{-1})$	$a_{\text{Cu}^{2+}}$ (nM)	$\gamma_{\text{Cu}^{2+}}$	[Cu <sup>2+</sup> ] (nM)	[CuCl <sup>+</sup> ] (nM)	[CuCl] (as % of total Cu)	[Cu <sup>2+</sup> ]
0.005	57	$1.9 \times 10^6$	0.0003	0.74	0.0004	0.000003	$6.0 \times 10^{-7}$	$7.2 \times 10^{-5}$
0.15	46	$1.0 \times 10^7$	0.0046	0.31	0.015	0.0017	$3.5 \times 10^{-4}$	$3.1 \times 10^{-3}$
0.35	46	$3.8 \times 10^6$	0.012	0.25	0.051	0.01	$2.2 \times 10^{-3}$	$1.1 \times 10^{-2}$
0.7	48	$1.7 \times 10^6$	0.028	0.23	0.12	0.048	$1.0 \times 10^{-2}$	$2.6 \times 10^{-2}$

$\nu_{\text{Cu}}$  = concentration of Cu specifically bound to humics; [] denotes solution concentrations;  $a_{\text{Cu}^{2+}}$  = free ion activity;  $\gamma_{\text{Cu}^{2+}}$  = activity coefficient of free ion.

Table 5. Selected WHAM output data for Cu binding experiments in NaCl solutions at pH ~4.5.

<i>I</i> (M)	$\nu_{\text{Cu}}$ (mol g <sup>-1</sup> )/10 <sup>-6</sup>	$\frac{\nu_{\text{Cu}}}{a_{\text{Cu}^{2+}}}$ (Lg <sup>-1</sup> )	$a_{\text{Cu}^{2+}}$ (nM)	$\gamma_{\text{Cu}^{2+}}$	[Cu <sup>2+</sup> ] (nM)	[CuCl <sup>+</sup> ] (nM)	[CuCl] (as % of total Cu)	[Cu <sup>2+</sup> ]
0.005	41	910	45	0.74	61	0.5	0.114	13
0.15	35	1000	35	0.31	110	13	2.67	24
0.35	24	500	47	0.25	190	40	8.38	41
0.7	15	290	53	0.23	230	88	18.7	48

Abbreviations as in Table 3.

median values of the ranges shown in Table 7. If the lowest  $K_o$  for Cu was used instead, the Mantoura ratios would be 16,000:8:1. Whichever Cu value is used, it is apparent that the relative binding strengths of Ca, Mg, and Co were approximately the same in both studies, but that the relative strength of Cu binding used in our study was much less. Therefore the greater effect of Ca and Mg competition on Cu binding, predicted in the Mantoura study, cannot be explained by the intrinsic binding strengths of the humics.

The concentration of humic binding sites in our experiments (see Table 7) was calculated from the added PHA concentration (10 mg L<sup>-1</sup>) and from the default total binding site concentration ( $4.94 \times 10^{-3}$  mol g<sup>-1</sup>) for humic acid contained in the WHAM database. The five sets of published proton binding data, used by Tipping (1993) to derive the default binding site concentration for humic acids, gave a range of 4.35 to  $5.25 \times 10^{-3}$  mol g<sup>-1</sup>. In the Mantoura study, the Cu concentration was  $1 \times 10^{-8}$  M but a number of trace metals were included in the model prediction, giving a total trace metal concentration of  $5.2 \times 10^{-8}$  M. Thus both studies employed a [Cu]:[binding site] ratio of 1:100, but the absolute concentrations of the two parameters were 50 times higher in our study (Table 7). The difference in the Ca-Mg-Cu competition effects between the two studies therefore could be related to the low availability of humic binding sites in the Mantoura study relative to seawater-Ca and -Mg concentrations, especially as other trace metals were also included in the model predictions. This possibility is discussed further in the following section.

### 5.3. WHAM Simulations under Realistic Estuarine Conditions

From the point of view of the likely effects on metal–humic binding, real estuaries differ most significantly from the exper-

imental conditions with respect to pH and the presence of additional inorganic ligands. The effect of low pH river waters on metal–humic binding is of particular interest. Whereas increasing ionic strength, cation competition, and inorganic speciation all serve to reduce metal–humic binding down an estuary, increases in pH may limit or even potentially reverse these effects.

WHAM was used in combination with its default database to predict the effects of ligand competition and variable pH on Cu and Co binding in two estuarine systems with contrasting river-water pH values. The default  $pK_{MHA}$  values were used in preference to the optimised values because the former are means derived from a range of published studies (Tipping, 1994) and therefore are likely to be more representative of humic acids in general. The compositions of the freshwaters were based on two soft-water Cumbrian lakes, Seathwaite Tarn (pH of 5.1) and Esthwaite Water (pH of 7.8). Intermediate estuarine compositions were calculated assuming conservative mixing of the various chemical components (Table 8), including alkalinity. The pH was calculated (Fig. 2a,b) assuming equilibrium with CO<sub>2</sub> in the atmosphere (0.00036 atm). The concentration of humic material was kept constant at 1 mg/L, a typical value for coastal waters (Libes, 1992). Cu and Co concentrations were set at  $3 \times 10^{-7}$  M, which is 1 to 2 orders greater than might be expected for these metals (Chester, 1990). Extreme trace metal concentrations and [M]:[binding site] ratios (~1:10) were chosen so as to maximise any competition effects from Ca and Mg for the humic ligand. The high concentrations are unlikely to affect the inorganic speciation as the inorganic ligands are present in great excess. Initial model runs included only one trace metal at a time.

Figures 2c and 2d demonstrate that Co speciation is generally dominated by the free metal ion and carbonate forms in

Table 6. A comparison between the metal humic binding, expressed as  $\nu_M/a_M^{2+}$ , in the end-member NaCl solutions predicted at fixed  $a_M^{2+}$  and pH, with the binding calculated under the experimental conditions used in deriving Tables 3–5. Values of variables used in the calculations are also shown.

Series	<i>I</i> (M)	pH	Total metal (nM)	$a_M^{2+}$ (nM)	Predicted $\frac{\nu_M}{a_M^{2+}}$ (Lg <sup>-1</sup> )	
					fixed $a_M^{2+}$ and pH	as in Tables 3–5
Co	0.005	7.68	510	3.2	$1.6 \times 10^4$	$2.5 \times 10^4$
Co	0.7	7.68	21	3.2	19	19
Cu, high pH	0.005	7.73	470	0.0003	$1.6 \times 10^8$	$1.9 \times 10^8$
Cu, high pH	0.7	7.73	6.0	0.0003	$2.0 \times 10^6$	$1.7 \times 10^6$
Cu, low pH	0.005	4.46	470	65.9	580	910
Cu, low pH	0.7	4.46	590	66.9	290	290

$\nu_M$ -concentration of metal specifically bound to humics;  $a_M^{2+}$ -free ion activity.

Table 7. A comparison of parameter values used in this and the Mantoura et al. (1978) studies.

This study		Mantoura et al. study	
[Cu]	$4.7 \times 10^{-7} \text{M}$	[Cu]	$1.0 \times 10^{-8} \text{M}$
[binding sites]	$5 \times 10^{-5} \text{M}$	[total metals]	$5.2 \times 10^{-8} \text{M}$
$\text{p}K_{\text{CaHA}}$	3.2	[binding sites]	$1 \times 10^{-6} \text{M}$
$\text{p}K_{\text{MgHA}}$	3.3	$\log_{10} K_o$ for Ca	3.6–4.1 <sup>a</sup>
$\text{p}K_{\text{CuHA}}$	1.5	$\log_{10} K_o$ for Mg	3.4–4.1 <sup>a</sup>
$\text{p}K_{\text{CoHA}}$	2.7	$\log_{10} K_o$ for Cu	8.0–10.4 <sup>a</sup>
		$\log_{10} K_o$ for Co	4.5–4.9 <sup>a</sup>

<sup>a</sup> Typical values, omitting outliers.

both estuarine simulations, in agreement with the predictions of Mantoura et al. (1978) and Tipping et al. (1998). In all three studies the predicted Co-humic complexation reaches a maximum, generally within the range 0.5 to 5% of the total Co, at or close to the freshwater end-member. In the relatively high pH system (Fig. 2d), the predicted percentage humic-Co decreases continuously down the estuary from 7% to 0.1% of the total Co. In the low pH system (Fig. 2c), humic binding in the freshwater end-member is decreased from 7% to 1.6% due to proton competition. In contrast to Co, Cu speciation is dominated by humic complexation in both estuaries (Fig. 2e,f). As with Co, however, humic binding is lower (84%) in the freshwater end-member of the low pH system (Fig. 2e), due to proton competition, and carbonate species are increasingly important with increasing ionic strength at both pH values. In the seaward end-member, 90% of the Cu is humic-bound and 10% occurs as carbonate species. To determine the contribution of Ca and Mg competition to this predicted decrease in Cu-humic binding in full-strength seawater, relative to that predicted and observed at 0.7 mol/L ionic strength in mixed salt solution (Fig. 1d), the seawater simulation was rerun with the  $\text{p}K_{\text{MHA}}$  for Ca and Mg set to 999 (i.e., effectively equivalent to no Ca and Mg binding). The result was to increase the Cu-humic binding from 90% back up to 96%, so that there does appear to be a competition effect under more realistic estuarine conditions but it remains small compared to that reported by Mantoura et al. (1978).

Additional modeling was undertaken to explore the effects of Co-Cu competition and the natural variability in humic binding. To examine competition, the estuarine simulations were rerun under the same conditions as above, except that Co and Cu were present together, each at their original concentrations ( $3 \times$

$10^{-7} \text{M}$ ). Natural variability was considered by running simulations for one trace metal at a time with alternative  $\text{p}K_{\text{MHA}}$  values for Co and Cu. The alternatives used were the extremes of the ranges for humic acid, and the default fulvic acid values, as contained in the WHAM database and shown in Table 2. The humic bound fractions predicted by these simulations are shown in Figure 3, together with the fractions predicted using the default humic acid  $\text{p}K_{\text{MHA}}$  values, shown previously in Figure 2. The most substantial differences are those linked to the natural variability of the humic substances. Ignoring the low binding in the Seathwaite (low pH) end-member, the predicted Cu binding decreased to  $\sim 70\%$  in seawater when the humic substances were assumed to be fulvic acid. For humic acid, Cu binding remained above 85% at all  $\text{p}K_{\text{MHA}}$  values. The predicted effect of humic variability was even greater for Co, especially with respect to the extreme  $\text{p}K_{\text{MHA}}$  values of 1.9 and 3.8. It is apparent that a value of 1.9 effectively changes the nature of Co from being a weak binding to a strong binding metal, more comparable to Cu (see Fig. 3a–d). The question then arises as to how representative the Co  $\text{p}K_{\text{MHA}}$  values are for humic acid in the WHAM database, especially as they are based on only three published datasets (Higgo et al., 1993; Tipping, 1993). The optimised  $\text{p}K_{\text{MHA}}$  value ( $\sim 2.5$ ) for Co obtained in the present study for a PHA and the previously reported correlation between  $\text{p}K_{\text{MHA}}$  values and the analogous values for lactic acid (Tipping and Hurley, 1992) both indicate that the default  $\text{p}K_{\text{MHA}}$  value of 2.7 for humic acid is the most representative. However, the above simulations do highlight the need for further high-quality measurements of metal–humic interactions for at least some metals. In comparison to the effects of natural variability in humic substances, the predicted competition effects between Co and Cu are small, even at the

Table 8. Composition of end-member waters used in the simulated estuary predictions of metal specification.

Component	Esthwaite	Seathwaite	Seawater
	(mM)		(M)
Na	250	193	0.488
Mg	60	33	0.0552
K	25	10	0.0102
Ca	265	28	0.0107
Sr	0	0	0.00009
Cl	280	212	0.569
$\text{NO}_3$	30	36	0
$\text{SO}_4$	115	42	0.0293
$\text{HCO}_3$	385	0	0.0024
pH	7.8	5.1	8.3



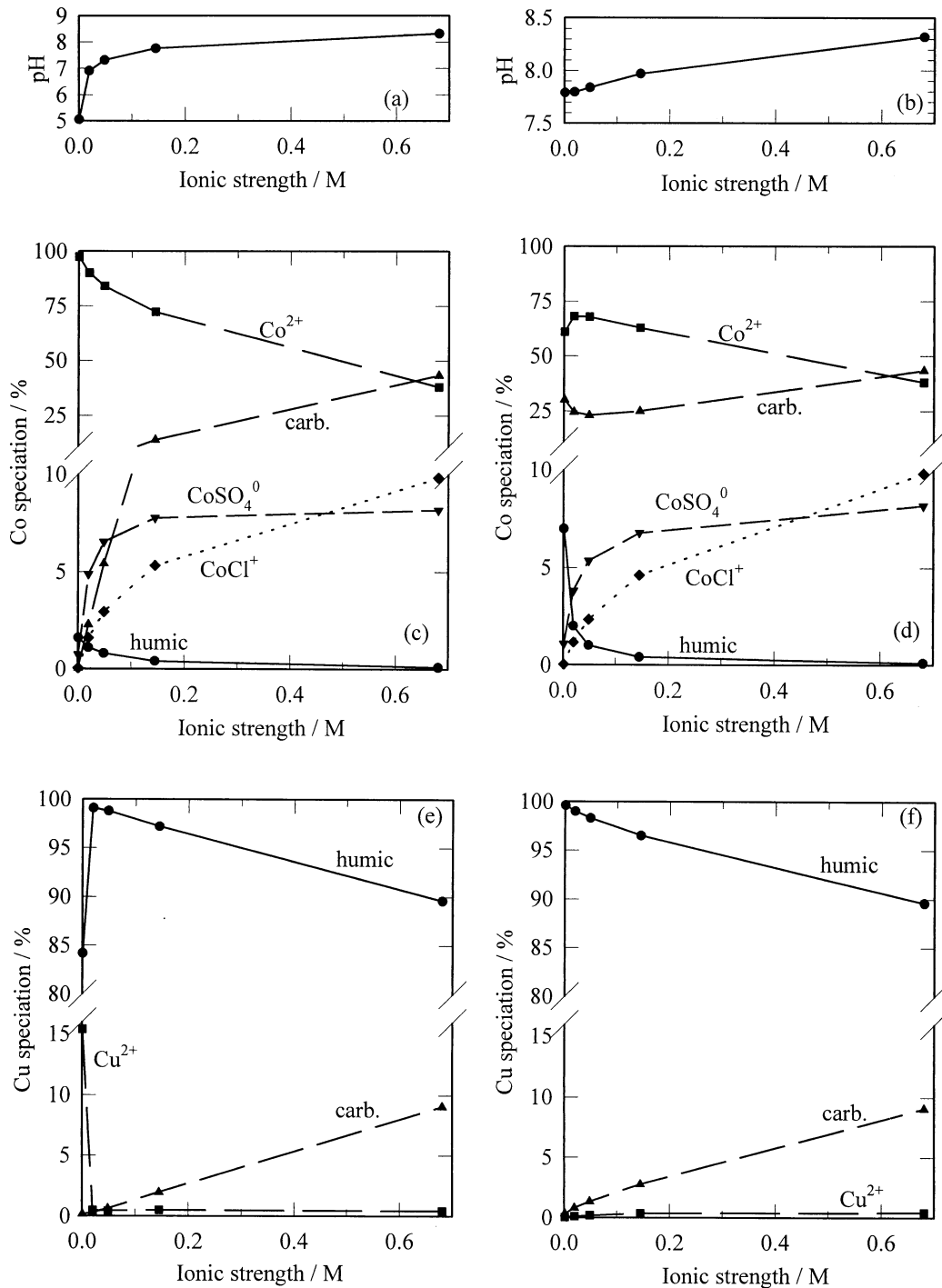


Fig. 2. WHAM predictions, using the default database, of pH, metal–humic binding, and inorganic metal speciation in simulated estuaries with low (a, c, e) and high (b, d, f) freshwater end-member pH values. With the exception of humic-bound M and the free metal ion, species are only included if >1% of the total metal at some point in the estuary. Combined carbonate and bicarbonate species are indicated by “carb.”

high metal:humic ratios used in the simulations. A small competition effect is predicted in the case of Co binding (Fig. 3a,b), but effectively none at all in the case of Cu (Fig. 3c,d), reflecting the contrasting binding strengths of the two metals.

Given the particular interest in this study of Ca and Mg

competition on Cu binding, further WHAM simulations were undertaken to explore the effects of variable Cu and humic concentrations on humic binding. These simulations used the default humic acid  $pK_{MHA}$  values and were run for full-strength seawater at more typical estuarine concentrations of total Cu

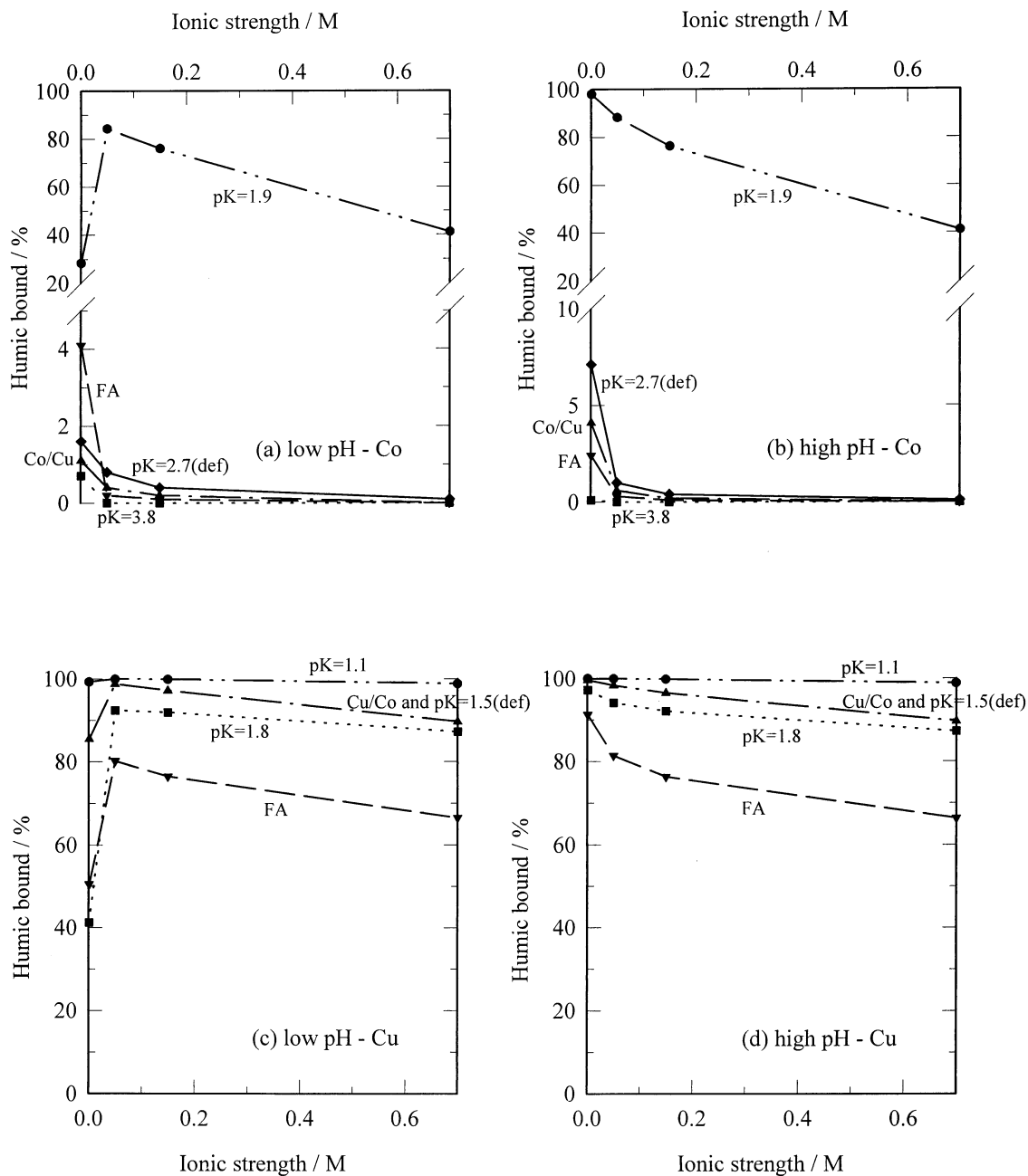


Fig. 3. WHAM predictions of metal-humic binding in simulated estuaries with low and high freshwater end-member pH values for humic acid at various assumed  $pK_{MHA}$  values, including the defaults (def), and for fulvic acid (FA), using the default  $pK_{MHA}$  values.

( $1 \times 10^{-8}$  M and  $1 \times 10^{-7}$  M), over a range of humic concentrations between 0.2 and  $1 \text{ mg L}^{-1}$ . One of these simulations ( $1 \times 10^{-8}$  M Cu and  $0.2 \text{ mg L}^{-1}$  humic acid) corresponds to the same concentrations of total Cu and humic binding sites as those used by Mantoura et al. (1978). Figure 4a shows that the predicted % Cu-humic binding decreases sharply once the humic concentration falls below  $\sim 0.5 \text{ mg L}^{-1}$  and it decreases with increasing total Cu concentration, as would be expected from the law of mass action. Figure 4b

shows the corresponding predictions of combined binding-site occupancy by Ca and Mg (i.e.,  $\nu_{Ca} + \nu_{Mg}$ ) and Ca, Mg, and Cu (i.e.,  $\nu_{Ca} + \nu_{Mg} + \nu_{Cu}$ ). The trends in  $\nu_{Ca} + \nu_{Mg} + \nu_{Cu}$  are again in line with mass action principles, but of particular significance is that the combined occupancy by Ca and Mg actually decreased with decreasing humic concentration, due to displacement by the more strongly binding Cu. In all cases, approximately half of the increased Cu binding was mitigated by decreased Ca and Mg binding, so that the predicted metal

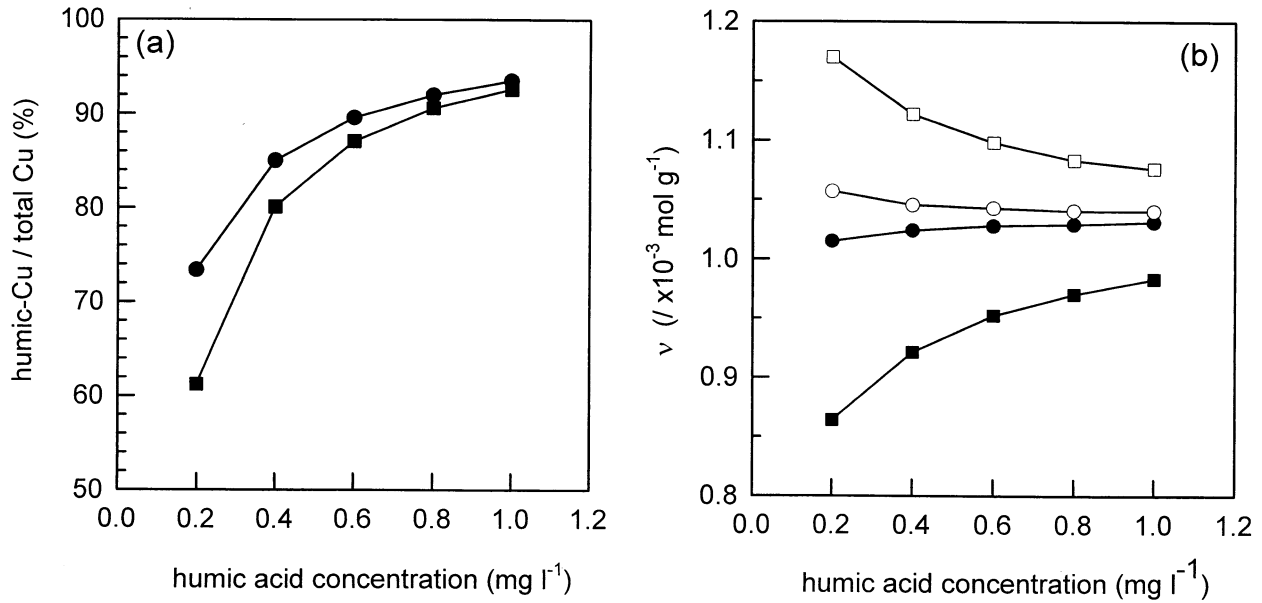


Fig. 4. WHAM predictions for seawater at total Cu concentrations of  $1 \times 10^{-8}$  M (circles) and  $1 \times 10^{-7}$  M (squares) and at various humic acid concentrations, using the default database, of (a) Cu-humic binding and (b) the combined binding-site occupancy by Ca + Mg (solid symbols) and Ca + Mg + Cu (open symbols).

competition effects were smaller than might have been expected. Because metal binding occurs preferentially at bidentate sites in the Model V framework (Tipping et al., 1998), a maximum level of binding site occupation by Ca, Mg, and Cu can be calculated from the predicted  $\nu$  values and the default concentration ( $4.94 \times 10^{-3}$  mol g<sup>-1</sup>) of binding sites for humic acid in the WHAM database (Tipping, 1994). The  $\nu$  values shown in Figure 4b correspond to a maximum site occupancy of 35 to 48%.

Both the experimental and modeling work undertaken in this study have indicated that Ca and Mg competition is not a major factor in influencing Cu-humic binding in estuaries. We suggest that the key factor as to why Mantoura et al. (1978) predicted a decrease in Cu-humic binding from ~100% to ~10% down estuary, linked to a >99% humic binding-site occupancy by Ca and Mg in seawater, is the electrostatic effect on the humic

molecule. In their model, Mantoura et al. (1978) included the humic material as an additional simple ligand with the assumptions that (1) the ratio of the activity coefficients of the humic ligand and the metal–humic complex was unity, and (2)  $K_o$  did not vary over the small pH range (~7.5 to 8.1) used in the modeling. Assumption 1 means that  $K_o$  is independent of  $I$ , implying that there is no electrostatic effect on the humic molecule. Thus the conditional binding constants measured at  $I = 0.02$  mol/L were applied universally.

The effects of assumption 1 on the WHAM predictions were assessed by means of the following steps: (1) WHAM was run at identical conditions ( $I = 0.02$  mol/L, pH = 8.0,  $1 \times 10^{-6}$  M humic binding sites, and  $1 \times 10^{-8}$  M Cu) to those used by Mantoura et al. (1978) in measuring their conditional metal–humic stability constants, with all WHAM parameters set to their default values. (2)  $P$  (see Eqn. 3) was then set to zero, so

Table 9. The combined occupancy of humic binding sites by Ca and Mg and the amount of humic-bound Cu predicted by WHAM for various waters at 0.2 mg L<sup>-1</sup> humic acid and  $1 \times 10^{-8}$  M total Cu, using different values of the electrostatic constant ( $P$ ) and  $pK_{MHA}$ ; Esthwaite Water predictions are also given for two other humic acid concentrations.

Water type	$P$	$pK_{MHA}$	$\nu_{Ca} + \nu_{Mg}$ (mol g <sup>-1</sup> )10 <sup>-3</sup>	$\frac{[\text{humic-Cu}]}{[\text{totalCu}]} (\%)$
0.02 M, pH = 8.0	default	default	1.136	96.2
0.02 M, pH = 8.0	zero	conditional	1.146	97.7
Seawater, pH = 8.3	default	default	1.015	73.4
Seawater, pH = 8.3	zero	conditional	2.20	59.2
0.02 M, pH = 6.9	default	default	0.854	98.3
Esthwaite, pH = 7.8	default	default	1.180	99.0
Seathwaite Tarn, pH = 5.1	default	default	0.111	63.2
R1 (Tipping et al., 1998), pH = 8.0	default	default	1.375	96.1
R2 (Tipping et al., 1998), pH = 7.5	default	default	1.247	96.8
Esthwaite, pH = 7.8, humics = 2 mg L <sup>-1</sup>	default	default	1.211	99.9
Esthwaite, pH = 7.8, humics = 10 mg L <sup>-1</sup>	default	default	1.208	100.0

that the exponential term in Eqn. (2) was equal to unity, and the model rerun with varying  $pK_{MHA}$  values for Cu, Ca, and Mg, until the amounts of specifically bound Cu and Ca + Mg were approximately the same as those found in step 1. The results of the final rerun (see Table 9) were achieved with  $pK_{MHA}$  values lower than the default values by 0.58. The new  $pK_{MHA}$  values are effectively conditional binding constants, directly comparable to those used by Mantoura et al. (1978). (3) WHAM was run for the seawater end-member using the new conditional binding constants for Cu, Ca, and Mg with  $P$  set to zero.

The above procedure resulted in a substantial decrease in the humic-bound Cu fraction in seawater to 59% and a marked increase in binding site occupancy by Ca and Mg to  $2.20 \times 10^{-3} \text{ mol g}^{-1}$ , when compared with the results for seawater using the default parameters (Table 9). These figures are significantly closer to the predictions of Mantoura et al. (1978), confirming the importance of the electrostatic term under estuarine conditions. The importance of the electrostatic effect under estuarine conditions is also supported by two other recent studies. Pinheiro et al. (1999) found that an increase in ionic strength from 0.02 to 0.1 mol/L produced a far greater lowering of Ca-fulvic acid binding than did metal competition from Pb, while Brown et al. (1999) reported a substantial decrease in binding with increasing ionic strength between Suwannee River fulvic acid and Cu.

#### 5.4. Predicted Ca and Mg Competition in a Range of Natural Waters

Table 9 shows that binding site occupancy by Ca and Mg is predicted, using the default parameters, to be greater at  $I = 0.02 \text{ mol/L}$  and a pH of 8.0 than in full-strength seawater at pH 8.3. This result is counterintuitive because seawater has approximately a 30-times greater concentration of dissolved Ca + Mg and half the proton activity (i.e., less proton competition). To explore this surprising result further, the occupancy of humic binding sites by Ca + Mg was also estimated at the same humic acid and total Cu concentrations for a range of low salinity waters, including the  $I = 0.02 \text{ mol/L}$ , pH = 6.9 water from the low-pH estuary simulation, used in this study, and the two hard-waters (R1 and R2) from the Humber catchment (U.K.), used in the modeling work of Tipping et al. (1998). Several general points emerge when the results are viewed as a whole (Table 9). Apart from Seathwaite Tarn water, in which proton competition was the predominant factor, there is a relatively small range ( $0.85$  to  $1.38 \times 10^{-3} \text{ mol g}^{-1}$ ) in site occupancy by Ca and Mg within the diverse range of water types considered. Furthermore, this range encompasses the values observed in seawater at varying humic and total Cu concentrations (Fig. 4b). It has been shown that the electrostatic effect on the humic molecule and ligand competition are important in limiting Ca and Mg binding in seawater. The same two factors, operating in reverse, are important in maintaining high Ca and Mg binding in nonacid freshwaters. Another important factor in maintaining binding, even in soft waters, is that the combined concentrations of Ca and Mg (e.g.,  $325 \mu\text{M}$  in Esthwaite Water; see Table 8) are far in excess of the total number of humic binding sites (e.g.,  $5 \mu\text{M}$  at  $1 \text{ mg L}^{-1}$  humic acid).

Table 9 also shows that, even with humic concentrations at the lower end of their natural range (i.e.,  $0.2 \text{ mg L}^{-1}$ ), humic-

bound Cu exceeds 95% except in seawater and in Seathwaite Tarn water, where the controlling factor is proton competition. Furthermore, for those waters with >95% humic-Cu, the exact percentage bound is not simply related to site occupancy by Ca + Mg. For example, the water (Esthwaite Water) with the highest humic-bound Cu has one of the highest occupancies by Ca + Mg. It follows that, in these examples, the amount of humic-bound Cu is influenced more by other factors, such as variations in Cu speciation, ionic strength, and hence the associated electrostatic effects on the various binding species. Therefore, competition from Ca and Mg does not appear to be a dominant factor in the humic binding of Cu in common natural waters. By analogy, it follows that the same will be true for other strongly binding metals (e.g., Al, Fe(III), Hg and Pb). This contrasts with the picture for weakly binding metals such as Co. Both the experimental and modeling studies have demonstrated the importance of Ca and Mg competition for Co. This competition is due to the greater similarity in the  $pK_{MHA}$  values of these metals, so that competition occurs even when binding site occupancy by Ca and Mg is relatively low. Other similarly weakly binding metals include Ni, Zn, and Cd.

The final observation concerning Table 9 is that the highest binding site occupancy by Ca and Mg, obtained with the default WHAM parameters, occurs in the slightly alkaline hardwaters (R1 and R2) followed by the slightly alkaline softwater of Esthwaite Water (Table 9). This high Ca- and Mg-occupancy is relatively unaffected by higher humic concentrations more typical of freshwaters (e.g., see the predictions for Esthwaite Water at humic concentrations of 2 and  $10 \text{ mg L}^{-1}$  in Table 9) because the combined concentrations of Ca and Mg are still substantially in excess of the total number of humic binding sites. The WHAM predictions therefore suggest that the most likely conditions in which Ca and Mg competition effects will be significant with respect to all metals are in fresh and brackish waters, and not seawater as intuitively expected.

Another surprising prediction concerning Ca and Mg competition is apparent in the estuarine simulations shown in Figure 3. In the case of Co, and in contrast to Cu, an increase in humic binding in the river water end-member was predicted in all simulations of the low pH estuary, apart from that involving a  $pK_{MHA}$  value of 1.9 (see Fig. 3a,c). The decrease in Cu binding has already been attributed to the low pH (5.1) of the river end-member, compared to that at higher ionic strengths (Fig. 2a), and the associated increase in proton competition. An insight into why an increase in Co binding is predicted is given in Table 9. As already noted, a relatively small range ( $\sim 0.8$  to  $1.4 \times 10^{-3} \text{ mol g}^{-1}$ ) in site occupancy by Ca and Mg was predicted for most natural waters using the default  $pK_{MHA}$  values. Of the waters considered in Table 9, only the river water end-member in the Seathwaite (low pH) estuary fell outside this range, with a low combined Ca and Mg occupancy of  $\sim 0.1 \text{ mol g}^{-1}$ . Therefore, the most likely explanation for the predicted increase in humic binding in the river water end-member is that, for a weak binding trace metal like Co, increased binding can occur due to substantially decreased competition from Ca and Mg under acid conditions. Ca and Mg binding are affected more by proton competition than Co because of their higher  $pK_{MHA}$  values (Table 7). In other words, the positive effect on Co binding of less Ca and Mg competition more than outweighs the direct negative effect of increased

proton competition. The same overall effect does not occur for a strong binding metal like Cu because the metal is not affected substantially by Ca and Mg competition in the first place, as demonstrated throughout this study.

This interpretation is supported by the predictions for Co at a  $pK_{MHA}$  value of 1.9 in Figure 3a. In this instance, the  $pK_{MHA}$  value corresponds to that of a strong binding metal, as previously discussed, and Co binding is predicted to decrease in the river water end-member, as with Cu (see Fig. 3a,c). It follows that this effect will be highly sensitive to the exact  $pK_{MHA}$  values for trace metals over a critical range (e.g., ~1.9 to 2.7 for humic acids) and, in the case of some trace metals (e.g., Co), to the natural variability in humic binding properties. These results highlight a major advantage of a general predictive model, such as WHAM, in that it is able to identify subtle competition effects of multicomponent equilibrium systems that are sometimes counterintuitive.

In this study, data interpretation and model predictions have been based on Model V as the humic submodel within WHAM. If trace metals are present at particularly low [metal]:[humic] ratios, it may be the case that better predictions would be obtained using Model VI (see Section 2), especially if the metals (e.g., Cu) have high affinities for the small number of strong binding sites that appear to present in at least some humic samples (Tipping, 1998). The main effect of using Model VI would be to increase the extent of humic complexation of some trace metals. Within the Model V framework, this effect is equivalent to lowering the  $pK_{MHA}$  values of some metals. The relative importance of the electrostatic effect is unlikely to be diminished by the choice of humic submodel, because Eqn. (2) operates similarly for the strong binding sites as for the monodentate and bidentate carboxyl and phenolic sites. The predicted Ca and Mg competition effects, if affected at all, will be less with Model VI because Ca and Mg have particularly low affinities for the additional strong binding sites (Tipping, 1998).

## 6. CONCLUSIONS

Laboratory experiments have demonstrated that Cu- and Co-humic binding decrease substantially with increasing ionic strength under typical estuarine conditions. The equilibrium chemical model, WHAM, provided a good description of the observed binding and indicated that the main factor causing the observed variations was the electrostatic (coulombic) effect on the humic molecule. WHAM also indicated that competition for humic binding sites by Ca and Mg in a wide range of natural water types, at typical humic concentrations, may be important for weak binding metals, e.g., Co, Zn, Cd, and Ni, but not for strong binding metals, e.g., Cu, Al, Hg, and Pb.

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